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NOTICE TO CONTRIBUTORS

General

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A brief summary is required, at the beginning of the paper. It should indicate the scope of the paper and give the principal results, and should be suitable for reproduction by abstracting journals as it stands.

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THE SELF-HEATING OF WET WOOL

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Summary

A study has been made of the tendencies for various grades of wet wool to self-heat under aerated adiabatic conditions, and comparisons made with the behaviour of wet wool in bales. Whereas 76°C is the highest temperature recorded for wet baled wool, it is demonstrated that adequately aerated wet wool can attain temperatures ranging up to 102°C under adiabatic conditions. An automatic apparatus capable of producing these conditions is described. The self-heating of wet wool up to 76°C is normally caused by bacterial metabolism, and it is shown that some thermophilic bacteria appear to be able to produce temperatures up to at least 79.4°C. Although all these bacteria utilise the wool fibre itself, their metabolic activity is greatly stimulated by the presence of grease, suint, scud, and other materials. When wet wool is kept hot (say 80°C or above) it degrades, and will then react chemically to cause further heating. This chemical heating reaction does not occur when the wool is completely dried. Aerated wool containing water in insufficient amount to saturate the interstitial atmosphere can also heat, probably because of the growth of moulds. All these effects are discussed in relation to the self-heating of other wet organic materials and to the known action of microbiological agencies on wool. The relation of this wet heating to the spontaneous ignition of wool is also discussed.

INTRODUCTION

When wet wool is assembled in large heaps or bales it can become hot, and in commerce this may lead to suspicions of incipient spontaneous combustion. The New Zealand Royal Commission to Inquire into Fires on Wool Ships (N.Z. Royal Commission 1907) arranged for an experiment in which water was added to wool before baling, and reported bale temperatures up to 60°C. Walker and Williamson (1957) baled wet slipe wool, and demonstrated bale temperatures up to 70°C. Walker and Williamson (1957) assumed that this heating was due to bacteria. Cases are also known of wet woolly sheepskins becoming hot

(N.Z. Royal Commission 1907; Anon. 1916; Green 1958); this heating has been shown to originate in the wool rather than in the skin, and is again believed to be due to the metabolic activity of bacteria (Green 1958). It is known that small pieces of wet woolly sheepskin can generate heat, since normal operation of the "cold" sweat pie process involves the self-heating of heaped pieces of woolly skin (Walker and Williamson 1957). Many other organic materials that permit free permeation of air are also known to become hot when assembled wet in large piles, the best known case being that of moist hay which sometimes rises to combustion temperatures (Browne 1933), although no completely satisfactory theory has been offered to explain the ignition mechanism. Other typical materials that self-heat when moist are jute (Finlow 1918; Obold *et al.* 1934; Bowes 1951); cotton (Anon. 1956); sawdust (Bowes 1956); straw (Carlyle and Norman 1941); stable manure (James *et al.* 1928); and seed grains (Sallans *et al.* 1944; Milner and Geddes 1946; Milner *et al.* 1947). Self-heating in these materials is generally considered to be due initially to microbiological activity. Laboratory experiments by various investigators have demonstrated maximum temperatures for such moist heating ranging from 55°C to slightly in excess of 100°C, although field experience with moist hay, moist sawdust, moist stable manure, and moist seed grains, has shown that actual combustion can sometimes occur. Investigations made of the self-heating of all these materials serve as a guide for the present investigation of self-heating in wool. In addition to wet wool, it is widely recognised in the wool trade that bales of wool packed damp (i.e., equilibrium RH less than, say, 95%) will sometimes heat. The self-heating of wet and damp wool has now been investigated to determine the causes, to establish the maximum temperatures that can be attained, and to see whether this wet heating might bear any relation to the spontaneous ignition of wool reported by Walker and Williamson (1957). The present experiments were carried out in a consequential series, and are therefore presented in each case together with the results.

Experimental Procedure and Results

General and Definitions

In all experiments the thermo-couples used were 0.032 in. diam. copper/constantan. Thermometers and thermo-couples were calibrated against NPL certified thermometers. Water baths used as thermostats were controlled by mercury contact thermometer $\pm$ 0.03°C. The nitrogen used contained about 50 p.p.m. of oxygen.

All wool used in the present work was New Zealand cross-bred. The "greasy" and "scoured" wools used had been sheared in the normal manner from living sheep, scouring being carried out commercially. The terms "slipe" and "pie" used in the text will be found defined by Crossland (1949) and Walker and Williamson (1957), and "Slipemaster" by Carrie *et al.* (1959). All slipe wool used was of "first grade", that is, it showed no visible contamination with sliping chemicals. The diethyl ether extract is referred to as "fat" throughout. Wool containing water in insufficient amount to saturate the interstitial

atmosphere is termed "damp" in the present work, and wool containing water in excess over that necessary to saturate the interstitial atmosphere is termed "wet". All moisture contents are expressed as "regain", that is, water content as a percentage of the oven-dry weight.

Experiment 1: Self-Heating of Wet Baled Pie Wool

Walker and Williamson (1957) describe experiments in which bales of first- and second-grade slipe wool were packed wet (35–95% regain), and attained temperatures of 50–70°C. The reduction in volume caused by "dumping" (tight pressing to reduce volume for shipping) appeared to inhibit heating in these experiments.

To discover how such heating would be influenced by the liability of pie wool to spontaneous ignition (Walker and Williamson 1957), neck trimmings from the flayed skins of freshly-slaughtered sheep and lambs were rinsed in cold water, and immersed in tanks of water at 35°C. The skin pieces were left in these tanks for three days, and the warm water changed daily. On the third day the pieces were rinsed in cold water, and wool was plucked from the rotted skin by hand. This wool was centrifugally hydro-extracted, and baled whilst still wet and cold. In this manner two bales of neck pie wool were prepared, one of sheep's wool and one of mixed sheep and lamb's wool, designated SP/75 and LP/75 respectively. Samples from each of these bales were analysed for water and fat content, and subjected (after drying at room temperature) to Walker and Williamson's ignition test (1957).

The two bales were placed in a room varying in temperature between 5°C and 13°C. Thermojunctions were inserted centrally in the bales, and temperatures kept under observation. Over the next few days bale temperatures rose above 70°C, one bale showing a slight lag

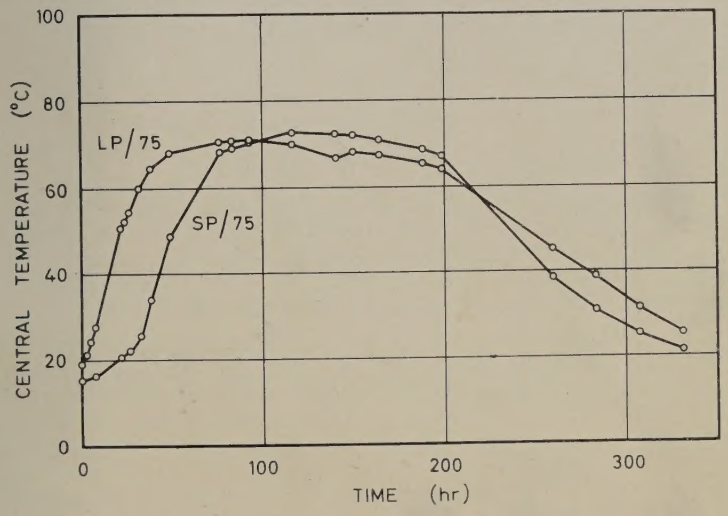

Fig. 1.—Self-heating of wet baled pie wool.

before commencing to heat (Fig. 1). The exposed surfaces of the bales remained cool to the touch at all times (say 25°C). After 15 days, the central bale temperatures had fallen almost to room temperature, and further samples were removed from central positions in the bales and air dried, and again analysed for fat content and tested for ignition. The relevant data in this experiment are summarised in Table 1. The wool remaining in the bales was discoloured and tender. The jute fabric enclosing the bales showed patches of brown markings that could readily be mistaken for scorch marks.

TABLE 1. SELF-HEATING OF WET BALED PIE WOOL

Bale designation	SP/75	LP/75
Commercial description	Sheep head pieces, tank-pied	Mixed sheep and lamb head pieces, tank-pied
Bale weight (net) (kg)	194	197
Bale size (cm)	70 × 70 × 100	70 × 70 × 100
Initial temperature (°C)	15.6	19.6
Peak temperature (°C)	72.7	71.3
Fat content (% on dry weight)	15.5	13.7
Moisture content (% on dry weight)	75.0	76.5
Max. temp. rise in ignition test	Fired	Fired
Time to reach max. temp. in ignition test (min.)	60	52
After Self-Heating Experiment		
Fat content (% on dry weight)	13.2	17.4
Max. temp. rise in ignition test (°C)	16	2
Time to reach max. temp. in ignition test (min.)	85	30

Experiment 2: Self-Heating of Wet Wool in Laboratory

In experiments with 10 lb parcels of wet slipe wool held in sealed vessels at 37°C, Walker and Williamson (1957) failed to obtain temperatures higher than 42°C; but they had no difficulty in demonstrating vigorous self-heating with whole bales. Moreover, their experiments demonstrated that self-heating appears to require access of air.

To provide better access of air, an experiment was carried out with a sack containing 9.3 kg cold wet fresh slipe wool (88% regain), roughly spherical in shape (38 cm diameter), and held on the laboratory bench (about 20°C) with a mercury-in-glass thermometer centred in it. Over three days the central temperature rose to 41.1°C and thereafter slowly fell. The surface was cool to the touch at all times. It was considered that, if thermal losses could be reduced, and an adequate quantity of air supplied without cooling the wool, higher temperatures

would be attained. A laboratory apparatus was therefore devised in which wet wool could self-heat, and an experiment was carried out to correlate the behaviour of wet wool in this apparatus with the behaviour of similar wool in a bale of commercial size.

A bale of cold wet sheep slipe wool was prepared as described by Walker and Williamson (1957). This bale was approximately 70 × 70 × 105 cm, and weighed 318 kg net (701 lb). The wool had a regain of 85%. A sample of this same wool was packed to a bulk density (wet) of 0.19 g/ml into a 10 l aspirator bottle, having the form of a cylinder 20.8 cm in diameter and about 30 cm high. The actual volume of the bottle used was 10,750 ml. The bottle was placed in a vigorously stirred lagged water bath, and was mounted in a weighted cradle designed to permit free circulation of water all around it. The water bath was 55 cm cube, fitted with a closely fitting split lid, and controlled in temperature by a thermo-regulator. Air was passed at 100 ml/min. through a sequence of three water-filled bubbling towers immersed in this thermostat, and into the bottle of wool. The temperature difference between the water bath and the wool centre was measured by a differential thermo-couple. The thermostat was adjusted to initial equality with the wool temperature, and the differential reading of the thermo-couple noted. Whenever the wool became appreciably hotter, the thermo-regulator was adjusted to increase the bath temperature, with the endeavour of keeping it always cooler than the wool. Fig. 2

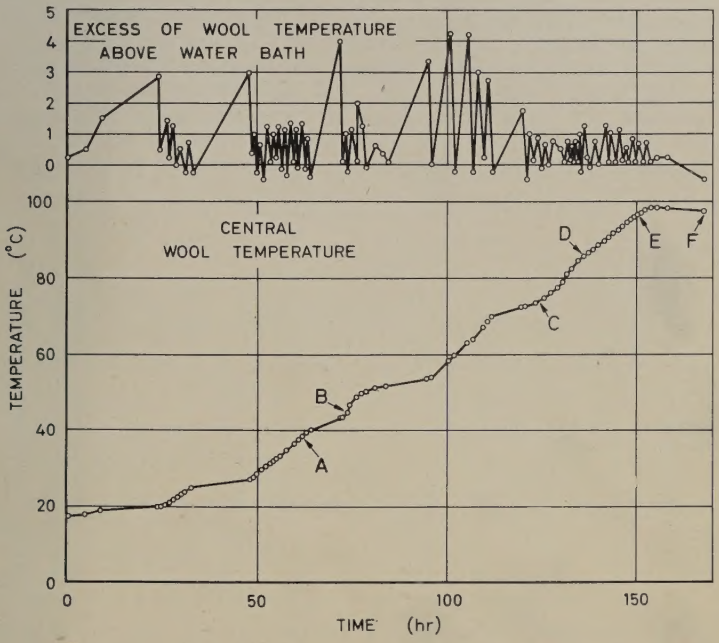

Fig. 2.—Self-heating of wet slipe wool.

shows the wool temperature, together with differences in temperature (on an expanded scale) between wool and thermostat. The exit gases were analysed, and in Table 2 are shown analyses of typical samples, removed at points A, B, C, and so on.

TABLE 2. ANALYSIS OF GASES FROM WET SLIPE WOOL
(See Figs. 2 and 3)

Sample Designation	Carbon Dioxide (%)	Oxygen (%)	Respiration Quotient
A	0.5	15.9	0.08
B	2.9	16.5	0.60
C	3.2	16.5	0.68
D	1.4	15.4	0.21
E	1.7	15.2	0.25
F	1.6	16.0	0.28
V	4.1	6.0	0.23
W	16.0	0.2	0.73
X	22.0	0.2	1.08
Y	15.0	2.4	0.77
Z	5.6	12.8	0.64
Air	0.0	20.9	—

Whilst this experiment was proceeding, the wet bale was stored in a room varying between 8°C and 13°C, and its temperature was measured on a chart recorder by six independent thermo-couples mounted in different positions in the bale. Although these reached temperature maxima at different times, the central temperature record is considered reasonably typical, and is here shown in Fig. 3. Gas samples were drawn from various parts of the bale, and typical samples (drawn from near the centre) are designated V, W, X, and so on, in Table 2. After point Z (Fig. 3), oxygen (containing about 0.5%

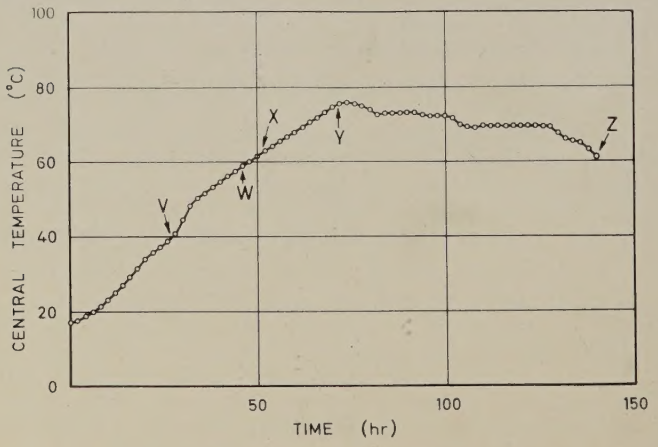

Fig. 3—Self-heating of wet baled slipe wool.

nitrogen) at a temperature of 70°c and saturated with water vapour was injected near the centre of the bale at a flow rate of 1 l/min. for four days. The temperature slowly fell at all parts of the bale during this time. At the conclusion of this experiment examination showed that up to 10 cm in from top and sides the wool was of a good colour, but the rest of the bale was rather brown and tender. The bottom layer of wool was grey-white in colour, and very rotten. Once again, brown markings were noticed on the fabric pack.

Measurements of pH were made by macerating a sample (free from sliping compound) with a minimum of distilled water. Before heating, the pH was 9.7; after heating in the bottle it was 7.7; and after heating in the bale it was 8.4. An ignition test (Walker and Williamson 1957) was carried out on the original wool (after air-drying), and found to rise in temperature by 8°c in 200 min. After heating in the bottle in the water bath a further sample was similarly tested, and heated 2°c in 60 min. Both these tests showed that the samples had a negligible tendency towards spontaneous ignition when dry.

Experiment 3: Development of Automatic Adiabatic Apparatus

Experiment 2 described above demonstrates that, under sufficiently adiabatic conditions, the provision of an adequate supply of air saturated with water vapour at a temperature a little below that of the wool, will permit the self-heating of wet slipe wool to take place in a mass of only 2 kg. To provide for automatic operation, the thermo-couple signal was connected to a galvanometer, deflecting a light beam on to a cadmium sulphide photo-conductive cell, and so actuating the bath heaters. This circuit is shown in Fig. 4, and components in Table 3. The signal on the galvanometer varied by

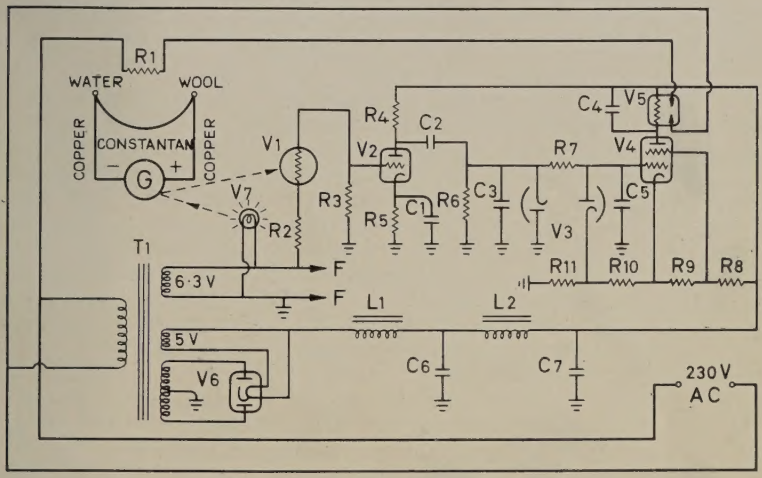

Fig. 4.—Control circuit for water bath. (See Table 3).

TABLE 3. COMPONENT VALUES IN FIG. 4

C_1	=	25 μ F, electrolytic, 25V
C_2, C_3, C_4, C_5	=	0.1 μ F, paper, 600V
C_6, C_7	=	16 μ F, electrolytic, 500V
G	=	Galvanometer, Cambridge "Spot", 10 ohms
L_1	=	10 henries, 100 MA, 60 ohms
L_2	=	30 henries, 100 MA, 190 ohms
R_1	=	Immersion heater, 40 ohms
R_2	=	15,000 ohms, 1 W
R_3, R_4	=	33,000 ohms, 1W
R_5	=	2,200 ohms, 1W
R_6, R_7	=	270,000 ohms, 1W
R_8	=	1,800 ohms, 10W, WW
R_9	=	3,300 ohms, 10W, WW
R_{10}	=	12 ohms, 3W, WW
R_{11}	=	470 ohms, 10W, WW
T_1	=	Power transformer, 380–0–380V, 100 MA; 6.3V, 4 amp; 5V, 2 amp.
V_1	=	Cadmium sulphide photocell, ORP 30
V_2	=	6 C 5
V_3	=	6 H 6
V_4	=	6 L 6 G
V_5	=	Sunvic 602V hot wire vacuum switch
V_6	=	5 V 4 G
V_7	=	Lamp, 6V, 2 amp.

approximately $\pm$ 2μ V about a stable mean value. This apparatus was free from the defects of similar systems previously described; these defects included very small temperature differential (Norman *et al.* 1941; Sallans *et al.* 1944; Gross and Robertson 1958), excessive random drift (Gross and Robertson 1958), lack of uniform performance at all temperatures (Milner and Geddes 1946; Bowes 1951), and unreliability in operation. To ensure that all rises in temperature were due to heating reactions in the wet wool, the apparatus was operated with the centre of the wool always hotter than the water bath by about 0.25°c $\pm$ 0.08°c (except in the case of Fig. 10—see Experiment 4 below).

The 10 l bottle was filled with wet wool (about 100% regain), and a differential thermo-couple connected between the water bath and the centre of the wool (both junctions were enclosed in insulating glass sheaths). The water bath was adjusted to a little below wool temperature, and the bath heaters placed under control of the galvanometer amplifier (Fig. 4). Air was passed as before through the humidifying train at 100 ml/min. (measured dry at room temperature), and the potential of the differential thermo-couple was monitored manually on a Diesselhorst potentiometer to check that the equipment was operating satisfactorily. Figs. 5–10 show typical time/temperature curves obtained in this way, selected as representative of a large number of experiments (See Experiment 4 below). In spite of the large quantities of water carried through this apparatus in the air stream during high-temperature operation, weighings of the samples before and after each experiment showed losses or gains of up to, say, 20 g in most cases. In some experiments carbon dioxide production was estimated by absorbing the exit gases in caustic soda solution,

followed by potentiometric titration. The rate of heat production in this apparatus can be calculated by assuming that the cylindrical bottle is equivalent to a sphere of slightly greater radius (about 11.3 cm), and by assuming that the slight heat losses due both to air flow and to thermal conduction down the thermo-couple leads can be ignored. The rate of heat production is represented by the sum of heat which causes a steady temperature difference between the centre of the wool and the water bath (cf. Walker and Harrison 1960) and of heat which causes a general increase in the wool temperature. In view of the desirability of expressing heat output on a dry wool basis, it is most convenient in writing an equation for heat output to express some parameters on a dry wool basis (e.g., specific heat) and some on a wet wool basis (e.g., thermal conductivity). As a rough approximation it can be assumed that the specific heat of the wet wool is a sum of the individual specific heats of wool and of water. The operative specific heat is that at constant pressure, but phase changes will have a negligible influence on specific heat under the present conditions (less than 3% up to 90°C). Then, assuming a spherical reaction vessel,

$$Z = \frac{6\,k\,t}{\rho\,r^2} + \omega\left(c + \frac{M}{100}c'\right) \quad \ldots\ldots\ldots (1)$$

where Z = rate of heat generation by wool on a dry weight basis (cal/sec/g),
k = thermal conductivity of wet wool (cal/sec/sq cm/cm/°C),
t = central temperature difference (wool hotter) (°C),
ρ = density of wool on a dry weight basis (g/ml),
r = effective radius of reaction vessel (cm),
ω = rate of temperature rise of wet wool (°C/sec),
c = specific heat of dry wool (cal/g/°C),
M = moisture content of wool (% regain),
c' = specific heat of water (cal/g/°C).

The difficulty in applying this formula is the unknown state of values for c and k for raw wool. The specific heat of dry wool can be taken as 0.325 cal/g/°C (Matthews 1927), with appropriate small corrections when grease is present. Moreover, under conditions approaching adiabatic, the accuracy with which k is known is relatively unimportant in the case of heat outputs greater than 10^{-4} cal/sec/g (dry wool). Transfer of heat in wet wool will probably take place by simultaneous diffusion of heat and of moisture. It is here assumed that, under the present conditions of experiment, wet slipe wool at a bulk density of 0.3 g/ml (wet weight) has an effective thermal conductivity ranging from 4×10^{-4} cal/sec/sq cm/cm/°C at 30°C, through 10×10^{-4} at 60°C, to 23×10^{-4} at 80°C, at regains between 80% and 120% (unpublished experiments). In this way, the rate of heat generation can be calculated at each temperature regardless of the amount of offset used on the light beam to operate the galvanometer

amplifier. These heat production figures are also shown in Figs. 5–10. Since these figures represent differentials of the heating curves, they show fluctuations, but the mean values are considered to be correct ± 20% or ± 25 × 10^{-6} cal/sec/g, whichever is the larger. The figures in the static experiments (Experiment 6 below) are less accurate because of doubt over the values for thermal conductivity, particularly at high temperatures. Calculations show that errors, due to flow of heat down the leads of the thermo-couple and to evaporation of water from the centre of the wool sample into the incoming atmosphere (humidified at thermostat temperature), make negligible contributions to this error. The water equivalent of the thermo-couple and its sheath similarly introduce insignificant errors.

Several investigators have designed automatic adiabatic reaction vessels in which moist organic materials could self-heat, and those who have attempted to calibrate such apparatus with an inert sample often remark on anomalous operation at different temperatures. Sallans *et al.* (1944) used a Dewar flask as reaction vessel and filled it with water for an inert sample. With the Dewar mounted in an air-bath thermostat, Sallans *et al.* (1944) adjusted the temperature differential to cause a temperature drop of 0.1°c/day in the sample, and found that the centre of the sample was then only 0.02°c hotter than the thermostat. It is unlikely that temperatures could be uniform to ± 0.02°c in an air thermostat. Milner and Geddes (1946) used a similar apparatus, and found that a temperature differential that caused the sample to cool at a rate of 0.2°c/day at 50°c, would cause the inert sample (water in this case) to heat at 25°c. They were unable to suggest an explanation for this effect. These two authors do not state if they bubbled humidified air through the water, but in any case water is not ideal as an inert sample, as evidenced by the difficulties described by Carlyle and Norman (1941). Bowes (1951) states that he initially used water as an inert sample, but then changed to jute fabric wetted with mercuric chloride solution to suppress biological activity. That all oxidation reactions were not suppressed is shown by Bowes' figures for CO_2 evolution from this sample of 0.4 × 10^{-8} g/sec/g (dry weight of jute). With this moistened jute Bowes (1951) found that heat losses from the Dewar flask appeared to depend on the temperature difference between the water bath and ambient temperature. The losses increased at higher temperatures. It would seem more likely that these losses were related to the third power of absolute temperature, and were due to the normal temperature coefficient of thermal conductivity for a Dewar flask. Bowes (1951) also found that cutting off the air flow reduced the heat losses almost to half. He recognised this effect as due to a combination of a saturation deficit in the incoming air and the very small size of the Dewar flask used (one pint). It is important that the incoming air be humidified in a succession of bubbling towers, since at high temperatures the latent heat of vaporisation will cause the water in the first bubbling tower to drop below bath temperature, leading to a small amount of evaporation in the second tower. In the present experiments very little make-up water was required in the second tower, and none in the third.

It was decided to check the performance of the present apparatus with an inert sample by making use of equation (1). Since the rate of heat generation is then zero, the two terms in the RHS of (1) are of opposite sign and should cancel out, and experiments can be performed with the signal on the differential thermo-couple maintained at a fixed amount positive (inert sample hotter); with the signal maintained at zero; and with the signal maintained at a fixed amount negative (inert sample colder). Since no sterilising agent is known that will not react with wool, and since it was considered unlikely that wet wool could be kept inert for the long times (several months) required for these calibration experiments, the apparatus was first assembled with dry wool. This had been rendered grease-free by extraction with acetone, and was packed to a bulk density (dry) of 0.080 g/ml, and ventilated with a stream of dry nitrogen at 100 ml/min. supplied through a heat exchanger. At temperatures ranging between 30°C and 40°C, with the wool maintained 0.25°C hotter than the water bath, it was found to cool at a rate of 3.4°C per day; and, maintained 0.20°C colder, it heated 2.8°C per day. Between 60°C and 70°C, with the wool maintained 0.23°C hotter, it cooled 3.7°C per day; and, with the wool 0.19°C colder, it heated 2.7°C per day. Between 90°C and 100°C, with the wool maintained 0.15°C hotter, it cooled 3.0°C per day; and, with the wool 0.21°C colder, it heated 3.3°C per day. At a temperature of 95°C, the mean signal on the differential thermo-couple was carefully adjusted to zero, and the wool temperature drifted less than 1°C per week over several weeks. By substituting likely values of thermal conductivity (Walker and Harrison 1960) and specific heat (Matthews 1927) in equation (1), these experiments show heat outputs from the inert sample of $0 \pm 5 \times 10^{-6}$ cal/sec/g dry wool, indicating the level of error inherent in the control system (with a dry sample).

The case of a wet sample is more complicated, in view of the possibilities of water transfer by the incoming gas saturated at thermostat temperature. This case was checked by using wet asbestos fibre as an inert sample, since it has a specific heat and a thermal conductivity very similar to wet wool. Commercial asbestos fibre was extracted with acetone in a Soxhlet, boiled with three changes of distilled water, cooled, and centrifuged free of excess water. It was then found to have a regain of 90%, and was packed into the 10 l bottle to a bulk density of 0.27 g/ml (wet weight). In this condition our experiments (unpublished) have shown it to have an effective thermal conductivity varying from 6.5×10^{-4} cal/sec/sq cm/cm/°C at 35°C, through 15×10^{-4} at 65°C, to 25×10^{-4} at 80°C. The bottle was placed in the water bath under automatic adiabatic control, and ventilated with nitrogen at 100 ml/min. humidified at the temperature of the water bath. Between 30°C and 40°C, with the centre of the wet asbestos maintained 0.24°C hotter than the water bath, it was found to cool at the rate of 5.3°C per day; and, when maintained 0.24°C colder than the water bath, it heated at the rate of 4.6°C per day. Between 40°C and 50°C, when maintained 0.24°C hotter, it cooled at the rate of 6.6°C per day; and, maintained 0.24°C colder, it heated at the rate of 5.6°C per day. Between 55°C and 65°C, when maintained

0.23°c hotter, it cooled at the rate of 8.8°c per day: and, maintained 0.23°c colder, it heated at the rate of 8.7°c per day. Between 75°c to 85°c, when maintained 0.22°c hotter, it cooled at the rate of 14.5°c per day; and, maintained 0.22°c colder, it heated at the rate of 14.4°c per day. At a temperature of 80°c, with the mean signal on the differential thermo-couple carefully adjusted to zero, the temperature rose 2°c over nine days. By substituting values of thermal conductivity (see above) and of specific heat (Matthews 1927) in equation (1), these figures show heat outputs from the inert sample of $0 \pm 25 \times 10^{-6}$ cal/sec/g dry asbestos. This higher error figure is due to the higher value of thermal conductivity of the wet asbestos. However, the experiments showed no evidence of anomalous behaviour at different temperatures. When the asbestos was removed from the bath after 95 days at temperatures ranging between 30°c and 95°c, it had lost only 14 g in weight, although nearly 2,000 g of water vapour had been passed through it in the ventilating nitrogen.

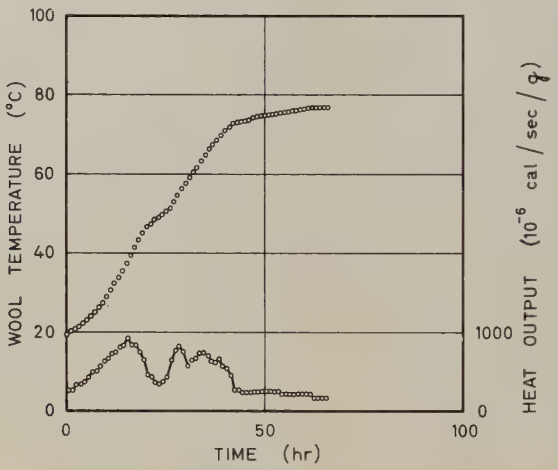

Fig. 5.—Self-heating of wet slipe wool.

Experiment 4: Adiabatic Self-Heating of Wet Wool

Figs. 5 to 10 illustrate results selected from many experiments as typical of those obtained with the automatic adiabatic apparatus:—

Fig. 5. Fresh wet slipe wool at 115% regain was packed into the 10 l bottle at 0.42 g/ml (wet weight), and temperature recorded automatically and manually. When the wool was at a temperature of 46.4°c the exit gas (at 80 ml/min.) contained 13.1% CO_2, 7.8% at 51.0°c, 6.2% at 74.5°c, and 0.3% at 76.2°c.

Fig. 6. Fresh wet slipe wool at 88% regain was packed into the 10 l bottle at 0.25 g/ml (wet weight), and temperature recorded automatically and manually. This experiment was halted at 92.0°c by a power failure. Analyses of the outflowing gases (at 100 ml/min.)

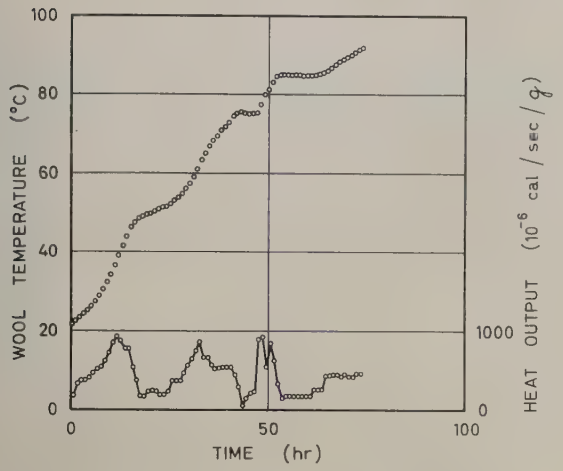

Fig. 6.—Self-heating of wet slipe wool.

showed 2.6% CO_2 at 75°C, 0.9% at 85°C, and 1.4% at 88°C. An ignition test (Walker and Williamson 1957) on the original wool gave a temperature rise of 5°C in 315 min.

The temperature rise from 76°C to 85°C in this experiment took place during the night, and, although the chart record shows no anomalies, it is conceivable that this rise might have been due to a transient defect in the apparatus. However, the rise from 85°C to 92°C took place in the day-time, and was closely supervised. This last rise undoubtedly represents a real generation of heat over this temperature interval.

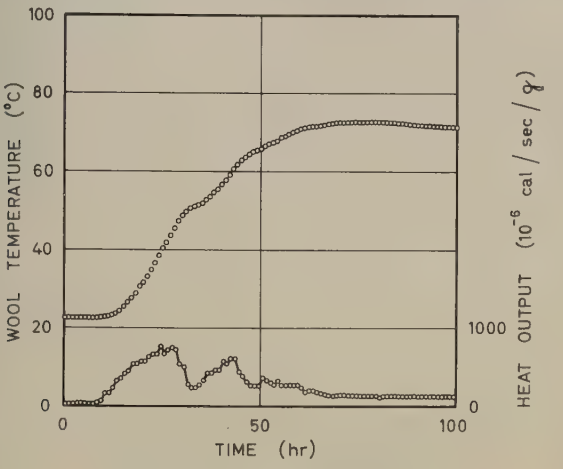

Fig. 7.—Self-heating of wet pie wool.

Fig. 7. Pie wool (manufactured six months previously) was wetted with tap water to 100% regain, and packed at 0.30 g/ml (wet weight). The temperature was recorded automatically and manually. An ignition test (Walker and Williamson 1957) on the original wool fired in 111 min. The ether extract was 12.0% on the dry weight.

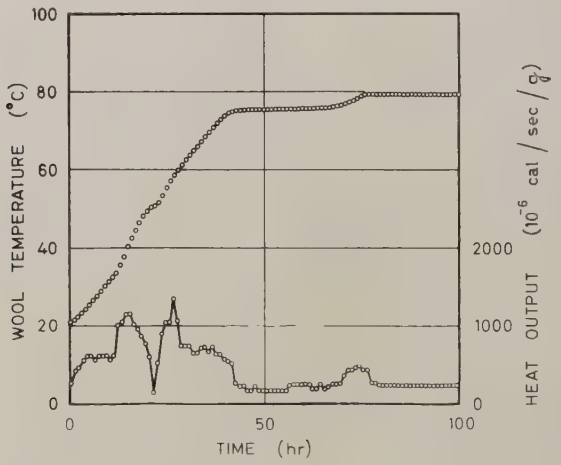

Fig. 8.—Self-heating of wet Slipemaster wool.

Fig. 8. Fresh wet Slipemaster wool at 135% regain was packed at 0.38 g/ml (wet weight) and temperature recorded automatically and manually. An ignition test (Walker and Williamson 1957) on the original wool gave a temperature rise of 9°C in 370 min. (A further experiment was carried out on this same sample—see Experiment 6 below).

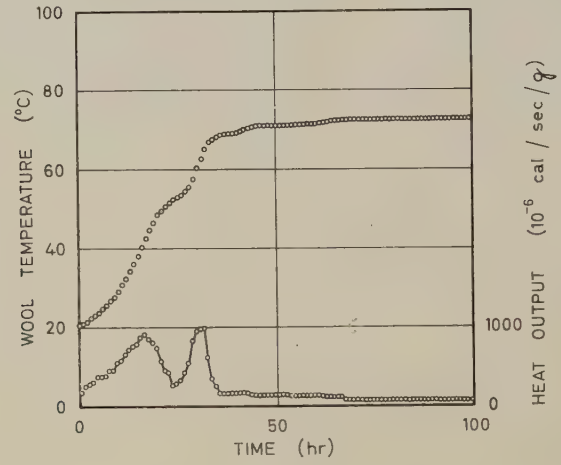

Fig. 9.—Self-heating of wet greasy wool.

Fig. 9. Greasy wool (sheared $3\frac{1}{2}$ years previously) was wetted with tap water to 100% regain, and packed at 0.25 g/ml (wet weight). The temperature was recorded automatically and manually. An ignition test (Walker and Williamson 1957) on the original wool gave a temperature rise of 1°c in 300 min. The ether extract was 8.3% on the dry weight.

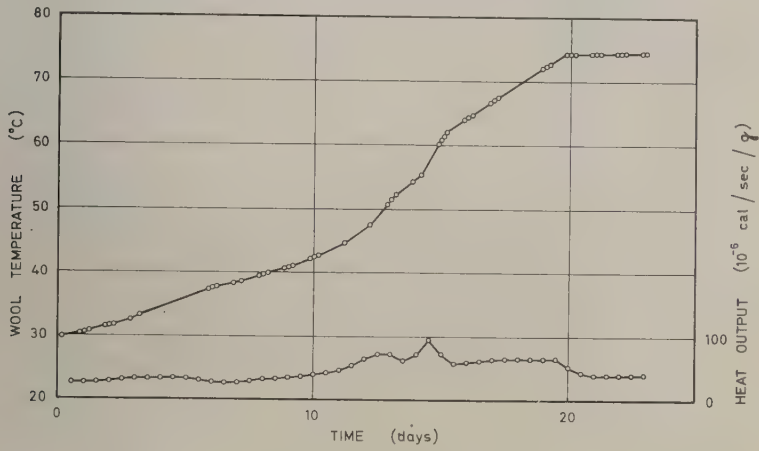

Fig. 10.—Self-heating of wet scoured wool.

Fig. 10. Scoured wool (commercially scoured three years previously) was wetted to 100% regain with tap water to which had been added 25 ml per l of water drips from a Slipemaster machine. It was packed to 0.20 g/ml (wet weight), and the temperature noted manually. Over 11 days at 20°c the sample failed to show any detectable heating. The temperature of the water bath and wool sample was accordingly raised to 30°c, and the galvanometer amplifier adjusted to operate on a thermo-couple signal of 6 μ V. The temperature then rose very slowly, as shown in Fig. 10. An ignition test (Walker and Williamson 1957) on the original wool gave negligible heating (< 1°c). The ether extract was 0.4% on the dry weight.

Experiment 5: Self-Heating of Damp Wool

The present authors have investigated many bales of wool found heated in the course of normal commerce, and have found that these frequently have moisture contents of the order of 18–25% (regain). When clean wool is in equilibrium with a saturated atmosphere at room temperature it will contain about 33% water (W.I.R.A. 1955), and clean wool containing less water than this will have a relative humidity of below 100% in its interstitial atmosphere. Since it is known that bacteria do not often metabolise actively at humidities below 90% (Haines 1937; see also Milner and Geddes 1946), it appears unlikely that the self-heating of these damp bales is caused by bacteria. It is known that moulds can grow at relative humidities

down to 75% (Galloway 1935), and can metabolise at temperatures up to about 55°C (Carlyle and Norman 1941; Milner *et al.* 1947).

To investigate the possibilities of self-heating at equilibrium relative humidities below 90%, a sample of slipe wool was selected from a bale which was stated to have been found slightly heated in commerce, although the wool was at room temperature when it came to our notice. This wool contained 23.2% water (regain), and when subjected to Walker and Williamson's test (1957) it heated only 3°C in 390 min. A measured volume of air was drawn through a large sample of this wool maintained at 20.8°C, and then through a magnesium perchlorate weighing train, and the relative humidity of this equilibrated air was found to be 82%.

The adiabatic apparatus described in Experiment 3 above was modified to suit this lower relative humidity. Instead of the incoming air being saturated in a water-filled bubbling train immersed in the water bath, it was saturated in a similar bubbling train immersed in a second water bath (dew point bath) also fitted with a heater and (in this case) a supply of cooling water. The gas pipe between the two baths was kept hot. By means of a thermo-couple and another galvanometer amplifier (as shown in Fig. 4) the dew point bath was adjusted in temperature to be a little cooler than the main water bath, so the differential thermo-couple between the two water baths was caused to generate a constant EMF at all temperatures. Because of the thermo-electric characteristics of copper-constantan (Roeser and Dahl 1938), the relation between dew point depression and relative humidity, and the equilibrium moisture characteristics of wool (W.I.R.A. 1955), this device ensures that, as the temperature rises above room temperature, the vapour pressure of the equilibrium water in the wool sample will become steadily greater than the vapour pressure of the incoming air, making certain that no heat could be generated within the wool sample by the latent heat of vaporisation of water. This wool sample was packed into the 10 l aspirator bottle to a bulk density of 0.19 g/ml (wet weight), and immersed in the main water bath at a temperature of 22.4°C. It was placed under automatic adiabatic control as before, and the dew point bath was adjusted to 18.9°C. This provided a relative humidity at the temperature of the main water bath of 81%, and an EMF on the differential thermo-couple between the two water baths of 140 μ V. The circuit of Fig. 4 was used to hold this voltage constant by automatically adjusting the temperature of the dew point bath. The temperature rise of the wool in this apparatus is shown in Fig. 11, together with the calculated heat output.

A further sample of slipe wool was selected from another bale found heated in commerce. When brought to our notice this bale was at a central temperature of 38°C, and contained 38.5% water (regain). The bale was placed in a controlled room at 24.4°C and 70% RH, and over the next three days it slowly rose in central temperature to 43.7°C, and then cooled. The surfaces remained cool to the touch at all times. A sample of wool was removed from the bale and conditioned to equilibrium with an atmosphere of 76% RH (at 21.1°C). It was then

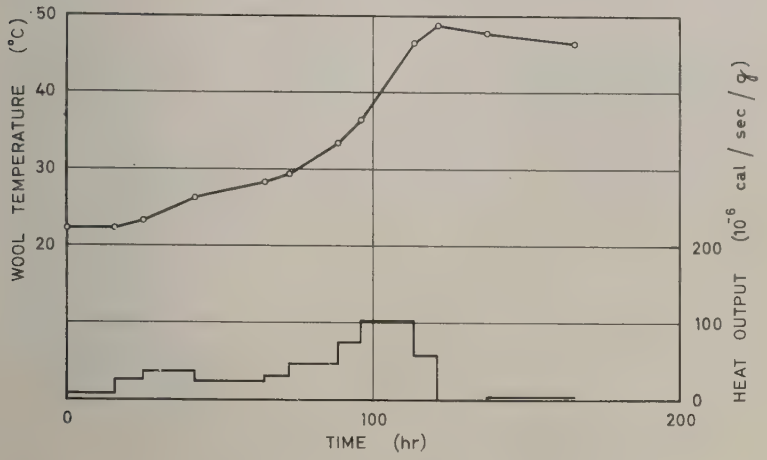

Fig. 11.—Self-heating of damp slipe wool.

placed in the adiabatic apparatus as before, at a density (wet weight) of 0.16 g/ml, with the dew point bath controlled to give an EMF of 185 μ V on the differential thermo-couple between the two water baths, providing a relative humidity of 76% for the incoming air. The main water bath was allowed to vary between 21°C and 28°C (as the laboratory air temperature varied), but no sign of heating could be detected over an 80-day period. It was therefore decided to condition the wool to equilibrium with a higher relative humidity. The main water bath was thermostatically controlled to 21.9°C, and the dew point bath to 18.4°C, and air was passed through at 1 l/min for 20 days to condition the wool to a relative humidity of 81%. At the end of this time the air flow was dropped back to 100 ml/min. and a known volume of the outflowing air was passed through a magnesium perchlorate weighing train to confirm the equilibrium RH. The water baths were then both put back under automatic control, with an EMF of 140 μ V on the differential thermo-couple between the two baths. Over the next 11 days the wool rose from 21.9°C to 46.7°C. At a temperature of 40°C it generated heat at a rate of 55×10^{-6} cal/sec/g (dry wool), and evolved CO_2 at a rate of 0.8×10^{-8} g/sec/g (dry wool). After removal from this experiment the wool had a moisture content of 22.5% (regain), and the pH of the water extract was 8.7. An ignition test (Walker and Williamson 1957) on the original wool gave a temperature rise of 9°C in 390 min.

Experiment 6: Experiments to Determine the Causes of Self-Heating in Wet Wool

The temperature rises shown in Figs. 5–10 have been chosen as typical examples, and it can be seen that variations exist in the maximum temperatures attained. Most wools ceased to heat appreciably at temperatures around 72–76°C. However, Fig. 8 shows a Slipemaster wool heating to 79.3°C, and Fig. 6 shows a slipe wool heating to 92°C.

This latter was still heating when the experiment was halted; and, although such behaviour is not common, it is by no means unique. The attainment of these higher temperatures appeared to be a characteristic of the wool sample concerned, and a sample of wet slipe wool (93% regain, wet density 0.24 g/ml) which rose to 72.6°C in 96 hours remained at a temperature varying cyclicly between 72°C and 67°C during the next 22 days. A series of experiments was therefore carried out to determine the causes of heating.

A sample of fresh wet slipe wool (100% regain, wet density 0.11 g/ml) was allowed to heat in the automatic adiabatic apparatus, and bacterial counts were taken at various times. Previous investigators have sometimes used a system of isolated satellite bottles around the main reaction vessel to provide samples for biological examination (e.g., Carlyle and Norman 1941; Milner and Geddes 1946), but it was considered preferable to use samples taken from the main reaction vessel for this purpose, since the population growth might not follow quite the same course in isolated vessels. The adiabatic apparatus described in Experiment 3 above was accordingly set up with a 2 l three-necked bolt-head flask as reaction vessel, with the neck of the flask rising above water level in the bath and closed with a rubber bung. This enabled small samples of wool to be removed (with sterile forceps) during the course of a temperature rise, with a minimum of interference with the experiment. Bacterial counts were made on these samples for both mesophilic and thermophilic bacteria, using plating techniques as described by Rothbaum (in press). These counts are shown in Table 4, together with the temperature of the wool at the time of sampling, and the time elapsing since commencing the experiment. In interpreting the thermophile counts it must be remembered that many thermophiles are spore-formers.

TABLE 4. BACTERIAL COUNTS IN SELF-HEATED WET SLIPE WOOL

Time (hours)	Wool Temperature (°C)	Mesophilic Bacteria (/g dry wool)	Thermophilic Bacteria (/g dry wool)
6	18	10×10^8	$< 10^3$
18	23	13×10^8	$< 10^3$
22	26	12×10^8	$< 10^3$
32	37	12×10^8	$< 10^3$
39	46	15×10^8	1×10^4
47	50	5×10^8	2×10^7
53	55.5	5×10^8	6×10^7
55	60	6×10^8	7×10^7
63	70	1×10^6	8×10^7
70	73	$< 10^3$	7×10^7

A sample of fresh wet lambs' slipe wool was obtained, having a regain of 99%. Analysis showed that the fat content was 10.7% (on the dry weight) and an ignition test (Walker and Williamson 1957) on the sample after air-drying gave a rise of 4°C in 390 min., indicating a negligible tendency towards spontaneous ignition. This wet wool

was packed into the 10 l bottle at 0.32 g/ml (wet weight). The bottle was placed in a water bath controlled at a temperature of 60.1°c, and air saturated with water vapour at thermostat temperature was passed through the wool at 150 ml/min. When the next observation was made 18 hours later, the centre of the wool had assumed a temperature 3.6°c hotter than the water bath, corresponding to a rate of heat generation of $1{,}200 \times 10^{-6}$ cal/sec/g (dry wool). Over the next 13 days this temperature difference steadily fell to 2.07°c (corresponding to 700×10^{-6} cal/sec/g) and remained stable at this value during the following 50 days. The rate of CO_2 production followed the same pattern, with a lag of about a day, falling from an initial peak of 5.9×10^{-8} g/sec/g (dry wool) to a value of 5.3×10^{-8} g/sec/g on the thirteenth day, and remained stable at this value during the following 50 days. Both heat generation and CO_2 production then began to diminish. After a total time of 75 days the wool had shrunk to about 25% of its original volume, but, since the weight of wet residue remaining was only 315 g lighter than the original weight, this meant that the wool was completely immersed in water, severely restricting access of air to the remaining wool fibres, so the sample was removed. In this experiment a total of 0.39 g CO_2 was collected for each g of dry wool.

To investigate the role of air in this thermophilic bacterial metabolism, a sample of fresh wet slipe wool (92% regain) was packed into the 10 l bottle at 0.31 g/ml (wet weight). When placed under automatic adiabatic control (aerated at 100 ml/min.), it rose from 24.8°c to 71.4°c in 40 hours, the rate of rise at 60°c corresponding to a heat output of $1{,}000 \times 10^{-6}$ cal/sec/g (dry wool). Over the following five days the temperature slowly fell from 71.4°c to 70.1°c. The inflowing air was then changed to nitrogen (again at 100 ml/min.). The temperature then fell at a steady rate over the next 114 hours to 44.0°c, and application of equation (1) yields a figure for the heat output of -7×10^{-6} cal/sec/g (dry wool) during this period. The air flow was then resumed and the temperature again rose to 71.0°c, and continued to range between that temperature and 69.3°c over the following two days, when the experiment was discontinued. At a temperature of 60°c the rate of this second rise corresponded to a heat output of 700×10^{-6} cal/sec/g (dry wool).

The cause of the maximum temperature attained by the Slipemaster wool in Fig. 8 was next investigated. A total of four separate samples of Slipemaster wool was obtained from the same works over a period of six weeks, and tested at regains ranging between 108% and 135%, and all heated to 79.4°c ± 0.3°c in this adiabatic apparatus. To investigate this heating, a sample of wool was removed from the reaction vessel of Fig. 8 after a further 36 hours at 79.3°c, and an attempt was made to grow any bacteria that might be present, in strongly aerated nutrient broth at high temperatures. No growth could be detected in the broth at temperatures higher than 68–70°c, which is the same temperature maximum found for the normal thermophilic bacteria present in wool. However, this one experiment is not regarded as conclusive. It was therefore decided to measure the

temperature coefficient of the heating reaction around 79.4°c, to determine whether heating is due to a biological or a chemical mechanism. For this purpose a fresh sample of Slipemaster wool at 128% regain was packed into the 10 l bottle at 0.36 g/ml (wet weight), and was found to rise from 21.6°c to 79.3°c in 86 hours. The temperature of the water bath was then lowered to 77.9°c, and controlled at that temperature. The centre of the wool then assumed a stable temperature 0.44°c hotter than the water bath, representing a rate of heat generation of 350×10^{-6} cal/sec/g (dry wool). The rate of CO_2 production at this temperature was found to be 2.1×10^{-8} g/sec/g (dry wool). The water bath temperature was then raised to 80.9°c, when the corresponding figures were 210×10^{-6} cal/sec/g and 0.7×10^{-8} g/sec/g. This demonstrates a strong negative temperature coefficient of reaction over the range 78–81°c.

Attention was next directed towards explaining the rise to 98.5°c demonstrated in Fig. 2, and the rise to 92°c demonstrated in Fig. 6. Rises of this magnitude are not usual with fresh wool, although they are sometimes noticed with samples that have self-heated and been cooled several times. A sample of fresh slipe wool (90% regain, 0.20 g/ml) was allowed to heat to 74.6°c (in 74 hours), and the water bath and wool sample were then cooled to 38°c. When again put under automatic adiabatic control the temperature rose to 96°c during 100 hours. The experiment was terminated at this point, and the rate of rise at 95°c showed that the sample was then generating heat at a rate of 440×10^{-6} cal/sec/g (dry wool) and evolving CO_2 at 1.7×10^{-8} g/sec/g (dry wool). A somewhat similar experiment was carried out with the Slipemaster wool shown in Fig. 8. After 2½ days at 79.3°c (see Fig. 8) the water bath was cooled to 62°c, and the wool rose to 79.4°c during the next two days, and remained at that temperature over another day. The water bath was again cooled (to 59°c), and the wool rose over the next seven days to 99°c. These experiments suggest that temperature rises above, say, 80°c may be initiated by some form of degradation of the wet wool that occurs at high temperatures. A sample of fresh slipe wool (95% regain, 0.24 g/ml) was accordingly allowed to self-heat to 72°c (in 44 hours), and was found to remain in the range 69–72°c for four days. The water bath was then raised in temperature to 84.6°c and thermostatically controlled at that temperature for two days, and finally lowered to 66°c. When the wool had come into thermal equilibrium at this last temperature (six hours) it was placed under automatic adiabatic control, and it then rose to 97.0°c in seven days. The water bath was then again placed under thermostatic control at 84.6°c, when the centre of the wool was found to be consistently 0.50°c hotter than the thermostat, corresponding to a rate of heat generation of 310×10^{-6} cal/sec/g (dry wool); and to be evolving CO_2 at the rate of 1.5×10^{-8} g/sec/g (dry wool). Over the next 14 days at 84.6°c the central wool temperature steadily rose to 1.05°c hotter than bath temperature (corresponding to 650×10^{-6} cal/sec/g), and the rate of CO_2 production similarly rose to 2.3×10^{-8} g/sec/g. The temperature of the water bath was then raised to 92.9°c and controlled thermostatically, when the centre of the wool assumed a temperature 1.8°c hotter than

the water bath (corresponding to about $1,400 \times 10^{-6}$ cal/sec/g), and the rate of CO_2 evolution was 2.9×10^{-8} g/sec/g. This experiment demonstrates a positive temperature coefficient of reaction over the range 85°C to 93°C.

To see if self-heating above, say, 80°C could be due to a chemical reaction initiated by degradation of the wool, an experiment was carried out under conditions where it is unlikely that bacteria could actively metabolise. Since most degraded wool (and all slipe wool) has a pH of 8 or above, alkaline conditions were initially chosen for this experiment. A sample of scoured wool (0.4% ether extractable matter) was wetted with $\frac{M}{10}$ $Na_2B_4O_7$ buffer (pH 9.8) to a regain of 100%. This was packed at 0.18 g/ml (wet weight) in the adiabatic apparatus, and thermostatically controlled to 80.6°C for four days. Heat output and CO_2 evolution steadily rose during this time, and, on the fourth day, the centre of the wool was 0.33°C hotter than the thermostat, corresponding to 125×10^{-6} cal/sec/g (dry wool). The rate of CO_2 evolution was then 0.6×10^{-8} g/sec/g (dry wool). The water bath was then placed under automatic adiabatic control (at this initial temperature of 80.6°C), and, over the next three days, the temperature rose to 98.3°C, the rate of generation of heat at 95°C being 380×10^{-6} cal/sec/g. A similar experiment was carried out with scoured wool wetted to 100% regain with tap water instead of borax buffer, and similar results were obtained at 80.6°C, although the reaction rates were about 20% lower.

A further experiment was carried out with scoured wool, which had been extracted in a Soxhlet apparatus with acetone. This wool was wetted with $\frac{M}{10}$ $Na_2B_4O_7$ buffer (pH 9.8) to a regain of 100%, packed in the 10 l bottle at 0.18 g/ml (wet weight), and thermostatically controlled at 84.7°C and ventilated with humidified air at 100 ml/min. The central wool temperature rose steadily above the water bath temperature until, at the end of seven days, it was 0.35°C hotter than the bath, representing generation of heat at the rate of 150×10^{-6} cal/sec/g (dry wool). At this stage the rate of CO_2 evolution was found to be 0.9×10^{-8} g/sec/g (dry wool). The ventilating air was then changed to a mixture containing 90% nitrogen, 10% hydrogen, and about 0.05% oxygen (as impurity). This gas was passed first over a catalyst to promote combination of the oxygen impurity with the excess of hydrogen, and then through a bubbling train containing vanadous sulphate solutions (Meites and Meites 1948). A flow of 200 ml/min. was used for five hours to purge the oxygen, and the flow rate was then reduced to 80 ml/min. The rate of heat generation showed little change, falling initially to 120×10^{-6} cal/sec/g, and then rising steadily over the following nine days to 270×10^{-6} cal/sec/g. The CO_2 evolution was found to continue at a steady rate of 0.4×10^{-8} cal/sec/g during this nine days in the N_2/H_2 atmosphere. To see if reaction with air could raise the temperature above 100°C the bath fluid was changed from water to a water/glycerine mixture (at the

same temperature), the incoming N_2/H_2 mixture was changed to air at 100 ml/min., and the bath immediately put on automatic adiabatic control. The temperature rose rapidly from 84.7°C to 95.7°C in $6\frac{1}{2}$ hours, representing a rate of generation of heat of 600×10^{-6} cal/sec/g (dry wool). At this temperature the incoming air was diverted past the humidifying bubbling train, and passed through the wool in a dry state. The wool immediately began to cool by evaporation of water (to a temperature of 94.6°C), so the flow of dry air was reduced to 15 ml/min. This was continued for two days but the wool continued to hold a temperature varying between 94°C and 95°C. The air flow was then cut off altogether, and the bath temperature rose over the next 10 hours to a temperature of 101.4°C, and remained at this temperature ($\pm$ 0.2°C) over the next four days. During this time the centre of the wool was consistently 0.18°C hotter than the bath. The water bath temperature was then lowered to 100.05°C and controlled thermostatically. With the air flow still cut off, the wool centre was found to be consistently 0.7°C hotter than the water bath over the next six days, when the experiment was discontinued. The wool remaining was brown, and very shrunken.

Discussion

The proclivity of bales of wet wool to self-heat is amply proven. The experiments carried out by the N.Z. Royal Commission on Fires on Wool-ships (N.Z. Royal Commission 1907) included bales of greasy fleece wool, scoured pieces, locks, slipe wool, and pie wool. These bales were packed with moisture contents varying from about 30% to 43% regain, and all became hot. Peak temperatures varied between 43°C and 60°C, but there was no obvious relation between peak temperature and regain. Walker and Williamson (1957) packed four bales of wet slipe wool at regains between 35% and 95%; these all heated, in general the wetter bales attaining higher temperatures. The three bales packed wet in the present experiments all became hot, and the peak temperature (76.0°C) recorded for the bale described in Experiment 2 above is the highest the present authors have measured or have seen reported for wet baled wool. Many descriptions of wet wool bales from trade sources speak of temperatures "too hot to bear the hand on", or of bales "which undoubtedly would have fired if they hadn't been opened". Such descriptions have often been accepted as evidence of incipient combustion in wet wool (e.g., N.Z. Royal Commission 1907), but it is obvious that a temperature of 76°C could well fulfill these descriptions if judged subjectively. A temperature of 76°C in wet wool is far removed from ignition. Trade sources often speak of "scorch marks" on the fabric packs of wool bales found wet and heated, and these may correspond to the brown patches found on the packs in the present experiments.

Experiments carried out with the automatic adiabatic apparatus show that the self-heating of wet wool that occurs in a bale can be duplicated on a laboratory scale by minimising heat losses and providing an adequate supply of air. Similarities can be seen between the shape of the heating curve for the bale of slipe wool (Fig. 3) and many of

the curves in Figs. 5–9. There are maxima in the heating rate at 40°C and 60°C, and a minimum at about 52°C. In self-heated bales of wet wool a temperature of 70°C can be attained in about 48 hours (Walker and Williamson 1957), and this is the case in Figs. 1 and 3 (after a slight induction period for bale SP/75). Wool allowed to self-heat in the automatic adiabatic apparatus also frequently attains a temperature of 70°C in about 48 hours. In the bale and in the adiabatic apparatus, at temperatures below, say, 75°C, when the rate of heat generation is larger than, say, 200 $\times$ 10^{-6} cal/sec/g (dry wool), the rate of temperature rise will be controlled by specific heat in both cases, rather than by thermal losses. The similarity of the curves therefore suggests that the causes of temperature rise to, say, 70–76°C are the same in both cases. At the air flow rate employed—100 ml/min. —the quoted rates of CO_2 evolution (and actual gas analyses) demonstrate that, during these peaks of activity, a major part of the oxygen in the laboratory experiment is replaced by CO_2. No attempt was made to avoid this, since the gas analyses of Table 2 show that similar conditions also exist in a bale. (It should be noted that all analyses of exit gases from wet wool are suspect in regard to CO_2 contents, and may be low to an unknown extent due to the possible simultaneous evolution of ammonia gas from the wool).

Carlyle and Norman (1941) investigated the self-heating of wet oat straw (300% regain) and observed similar features of maxima in the rates of heating at 40°C and 60°C, and a minimum at 52–55°C. In their experiments, wet straw typically reached a maximum temperature of about 70°C in about 44 hours. Carlyle and Norman (1941) concluded that heating was due to bacterial metabolism, and that 40°C and 60°C represent temperatures of maximum activity for mesophilic and thermophilic bacteria respectively. The bacterial counts in Table 4 show that below, say, 55°C self-heated wet wool has a large mesophilic population and above 50°C a large thermophilic population. It is generally agreed (e.g., Burgess 1954; Crewther 1955) that wet wool provides an adequate medium for bacterial growth. Crewther (1955) has pointed out that many micro-organisms attacking wool cause a rise in pH, and that this is an important factor in initiating attack on the wool fibre. Thermophilic bacteria of Allen's groups 1 and 3 (Allen 1953) prefer media of high pH. Rothbaum (in press) maintained samples of aerated wet wool at fixed temperatures, and demonstrated that mesophilic bacterial activity in wet slipe wool (80% regain) is capable of producing at least 1,500 $\times$ 10^{-6} cal/sec/g (dry wool), and thermophile activity 600 $\times$ 10^{-6} cal/sec/g. This mesophilic heat output is somewhat greater than has been found in the present work, and this is probably due in part to the more vigorous aeration used by Rothbaum (in press) and in part to the greater opportunity for bacteria to make best use of their environment when the temperature is not changing rapidly. This latter effect is less noticeable with thermophiles than mesophiles, since the generation time is much shorter. When a sample of wet slipe wool was maintained at a stable temperature of 60°C the thermophilic heat output was much higher, and was then comparable to the peak figure of 1,500 $\times$ 10^{-6} cal/sec/g (dry straw) obtained by Carlyle and Norman (1941) for wet oat straw at the same temperature. Rothbaum (in press) showed

that, in wet slipe wool at 50°c, the heat output per bacterial cell is lower than at any other temperature between 25°c and 65°c. This probably explains the shape of the temperature/time curves obtained in the present work, and also the shapes of curves described for many other wet materials whose self-heating is considered to be of bacterial origin, e.g., wet oat straw (Carlyle and Norman 1941), wet palm kernels, and wet jute (Bowes 1951). It is a more likely explanation for this phenomenon than Bowes' (1951) theory of endothermic reaction. It can therefore be concluded that the self-heating of wet wool to, say, 75–76°c is due to the activity of mesophilic and thermophilic bacteria. The drop to zero output of heat when the air atmosphere was changed to nitrogen (Experiment 6 above) demonstrates that these thermophiles are aerobes.

Fig. 10 shows that wet scoured wool (0.4% ether extractable matter) generated less heat over the range of 30–75°c than is typically generated by wools containing a greater content of impurities. Although this sample was inoculated with bacteria, it is doubtful if inoculation was necessary for heating to take place. Crewther (1955) has pointed out that micro-organisms which (when provided with minimal salts requirements) are unable to degrade solvent-scoured wool, may yet be able to initiate proteolysis in the presence of suint and other materials. Crewther (1955) remarks, however, that commercial experience shows that wet scoured wool is susceptible to bacterial attack. The present results confirm that ether-soluble materials assist bacteria to attack wool, but suggest that differences in activity would be mainly in degree. Green (1958) also reported less heating in wet scoured woolly sheepskins, but quoted laboratory experiments as showing that wet scoured wool would not heat. Rothbaum's demonstration (in press) that acetone-extracted wool wetted to 80% regain with distilled water (and seeded with thermophiles) will support about one tenth the thermophilic population normal in equally wet raw wool at 60°c disposes of Crewther's speculation (1955) that solvent-scoured wool might be appreciably more resistant to microbiological attack, at least so far as thermophiles are concerned. Fig. 10 shows anomalies in the temperatures of maximum and minimum heat output for this present sample of scoured wool, and it is not known if these would be confirmed by further experiments.

When fresh wet slipe wool was rapidly raised to a temperature of 60°c and maintained there (Experiment 6), it showed an immediate and vigorous exothermic reaction. This is considered to be due to the activity of thermophilic bacteria, and demonstrates that these bacteria do not require the prior activity of mesophiles for their growth. Other investigators have also carried out experiments that demonstrate this (Carlyle and Norman 1941; Rothbaum in press). This experiment was continued for 75 days, and, during most of this time, the bacterial activity continued at a high level. It is probable that the final diminution of activity was due to physical collapse of the wool fibres, with consequent restriction of the supply of air to the bacteria, rather than to exhaustion of the available nutrients. In this connection Allen (1953) has drawn attention to the significance of the reduced

solubility of oxygen in water at high temperatures in relation to thermophilic activity. In this experiment the measured evolution of CO_2 accounts for 0.105 g of carbon for each g of dry wool present. This is more carbon than is represented by the ether-soluble material (about 0.085 g carbon per g of dry wool). There can be no doubt that a major part of the bacterial nutrient was derived from wool proteins rather than from the grease and suint.

In attempts to prove that self-heating of wet organic matter is due to microbiological activity, the technique most commonly recommended is to demonstrate that sterile samples of the material do not heat, and then to induce heating by inoculation with pure cultures or with cultures prepared from the original materials. This technique has been widely used for materials such as hay, straw, cracked maize, and oil seeds. Another technique sometimes used is to carry out a blank experiment with material to which has been added an antiseptic agent (e.g., mercuric chloride) and demonstrate that no heating occurs (e.g., Bowes 1951). Neither of these techniques can be applied unequivocably to wool, since no means is known of sterilising wool without altering it chemically, and no antiseptic agent is known that will not react with (or modify) wool. Crewther (1955) has pointed out that much of the work on the action of pure strains of micro-organisms on wool is suspect because the sterilising techniques employed would increase the susceptibility of wool to proteolysis and therefore to attack by proteolytic organisms. This is particularly a problem in view of the known difficulties in sterilising thermophiles (Allen 1953). Haldane and Makgill (1923) have suggested an alternative technique to distinguish between chemical and biological oxidations. They claimed that a respiration quotient (i.e., ratio of CO_2 evolved to O_2 absorbed) of less than unity provides evidence of chemical oxidation; whilst, in biological oxidations, the respiration quotient is always about equal to, or above, unity. This criterion has been employed by other investigators (e.g., Hoffman 1935). There is no doubt that respiration quotients as low as 0.1 or 0.2 are common in the oxidation of unsaturated fats (Ellis 1936; Burgoyne and Thomas 1951; Walker and Harrison in press). However, there is little evidence that respiration quotients are always unity or above in biological oxidations. Bowes (1951) reported quotients around 0.7 in the fermentation of both palm kernels and greasy jute bagging; and, although quotients as high as unity were observed in the present investigation (particularly at very high CO_2 concentrations), quotients as low as 0.1 or 0.2 were also measured under conditions where there is no reason to suspect simple chemical oxidation (Table 2). Although Hoffman (1935) attempted to use the criterion of respiration quotient to distinguish between biological and chemical oxidation, the results he presented do little to support the theory. Milner and Geddes (1946) demonstrated systematic variations of respiration quotient with temperature, but their results show quotients above and below unity with both biological and chemical heating. It therefore seems that no reliance can be placed on respiration quotients to distinguish between chemical and biological oxidation. It is doubtful if any more reliance can be placed on Haldane and Makgill's other suggestion (1923) that chemical oxidation decreases

with time whilst biological oxidation continues, in view of the complications of order of reaction and difficulties with bacterial food supply and the possible production of toxic metabolic products. Better criteria are provided by positive correlation with biological growth, and by investigation of the effects of temperature and of oxygen partial pressure on the oxidation.

It is commonly considered that the maximum temperature at which thermophilic bacteria can remain active is about 75–76°c (Edwards and Rettger 1937; Bowes 1951). In many of the present experiments, generation of heat and evolution of CO_2 both dropped to a low level at this temperature. A reading of 76°c also represents the highest temperature reported for wet baled wool. However, in the four experiments with Slipemaster wool (typified by Fig. 8) heating continued to 79.4°c ± 0.3°c and halted there. No success was obtained in one attempt to culture from this wool thermophilic bacteria having a growth temperature higher than 70°c in aerated nutrient broth. However, this experiment is not regarded as conclusive, since 70°c was also found to be the maximum temperature of growth for the normal thermophiles present on wool, and yet the present work shows that these can metabolise in wool at temperatures in excess of 70°c (see also Rothbaum in press). It is also relevant to note Rothbaum's demonstration (in press) of the difficulties in finding optimum conditions for thermophilic growth. The strong negative temperature coefficient of reaction demonstrated for Slipemaster wool over the range 78°c to 81°c is considered to be proof that a major part of the heat evolution in this sample of Slipemaster wool at 78°c is not chemical in nature. It is considered that the heating of these four samples of wool to 79.4°c was caused by a strain of thermophilic bacteria able to metabolise at these higher temperatures. These four samples of Slipemaster wool were all obtained from the same source over a six-week period, and it seems likely that all may have contained the same strain of thermophile. Various investigators have carried out experiments at 80°c on the assumption that no bacterial action is possible at this temperature (e.g., Hoffman 1935). Others have considered that a demonstration of self-generated temperatures in wet material of only a few degrees above 75°c would provide evidence of the possibility of heating to ignition (e.g., Bowes 1951). It is evident that acceptance of these criteria will need to be revised in the light of the present experiments.

Of the experiments with samples of fresh wet slipe wool in the adiabatic apparatus, some heated right up to 92–99°c. It is considered that living organisms would be unable to metabolise at temperatures above 90°c. Immediate self-heating to these temperatures was observed only with slipe wools, and this may be due to the high initial pH of such wools, caused by residues of sliping chemical (Crossland 1949). However, this circumstance may have little significance, since the majority of the present series of experiments were made using slipe wool. Self-heating to temperatures approaching 100°c was more common when wools had been exposed wet to high temperatures for several days, and in these circumstances it occurred with wools other

than slipe. The production of ammonia (or more strictly ammonium bicarbonate) causes all wet degraded wools to be high in pH (say, 8–9). The two experiments with scoured wool wetted with borax buffer and heated at 80.6°c and 84.7°c in air (Experiment 6 above) are considered to be free from possibilities of any appreciable living oxidation, in view of the demonstrated low heat output of normal mesophiles and thermophiles on this same scoured wool at lower temperatures (Fig. 10). After several days' heating, both these wools were generating heat and evolving CO_2 at rates comparable to other samples of degraded slipe and Slipemaster wool at similar temperatures. With both these scoured wools the generation of heat and the evolution of CO_2 increased with time, and showed positive temperature coefficients of reaction. These characteristics were also shown by slipe and Slipemaster wools when damaged by wet heating (Experiment 6). The positive temperature coefficient demonstrated up to about 95°c suggests a chemical reaction rather than the metabolism of bacteria. From the present results it cannot be definitely concluded whether or not this chemical reaction is oxidation. The generation of heat continued undiminished when the air atmosphere was changed to a nitrogen/hydrogen mixture (Experiment 6), although the rate of CO_2 evolution was reduced. No analyses were made of the N_2/H_2 mixture flowing over the wool (except for CO_2 content); but either the catalyst alone, or the two-stage vanadous sulphate solutions alone, should have been capable of reducing the oxygen content to a negligible level. The vanadous sulphate solutions maintained their colour throughout the experiment. It is relevant to note the difficulties described by Bottomley *et al.* (1950) in reducing the atmosphere over mouldy corn below 0.1% oxygen by using acid chromous sulphate solutions. The present results suggest that there is an exothermic reaction occurring which either is not oxidation or is an oxidation reaction of a very low (perhaps zero) order with respect to oxygen partial pressure. This reaction increases with time (i.e., is auto-catalytic). It is here suggested that long-continued wet heating at a high pH causes the wool molecule to break into smaller units, and that either this reaction is exothermic or that the fragments then oxidise with a very low reaction order with respect to oxygen partial pressure. It is considered that the present experiments show that this reaction is restricted to wool with an appreciable water content (say, over 20%), and therefore could not raise the temperature of wool appreciably above, say, 105°c at atmospheric pressures.

Several other investigators have demonstrated temperature rises above 75°c with moist organic materials under adiabatic conditions. Milner and Geddes (1946) observed rises to 100°c with damp soya beans; but Milner *et al.* (1947) found it necessary to modify the characteristics of their apparatus to enable a sample of damp wheat to heat to 94°c. No investigator has yet been able to demonstrate a rise of this magnitude with hay in a laboratory apparatus, in spite of the fact that damp hay is known sometimes to ignite spontaneously in agriculture (Browne 1933). It is noteworthy, however, that Hoffman (1935) and Roethe (1937) have both reported maximum temperatures of 88°c in field experiments with large masses of hay. Milner and Geddes (1946) demonstrated that damp sterile soya beans can generate

heat at temperatures of 76°C and above, and Milner *et al.* (1947) suggested that this heating is probably caused by oxygen attack on the unsaturated oils that soya beans are known to contain. To investigate this question of oxidation of unsaturated oils, an experiment was carried out in which soya beans were lightly crushed between glass platens and subjected (under dry sterile conditions) to ignition tests, both before and after crushing, to see if crushing would have any effect on the rate of exothermic oxidation. The beans were packed into a 2 l flask, and dried in a stream of dry CO_2 in an oven at 100°C with cotton wool lining (cf. Walker and Williamson 1957). When the CO_2 flow was changed to oxygen, the undamaged beans were found to undergo a negligible temperature rise (1°C), but the crushed beans rose to 220°C (at the centre of the flask) in 11 hours, and the incipient fire was quenched by readmission of CO_2. Table 5 summarises

TABLE 5. Ignition Tests on Oil Seeds

Seed	Dry Bulk Density (g/ml)	Ignition Tests (Oxygen at 100°C)	
		Maximum Temp. Rise (°C)	Time to Reach Max. Temp. (h)
Whole soya beans	0.65	1	55
Crushed soya beans	0.48	120 plus	11
Whole wheat	0.76	2	42
Crushed wheat	0.54	4	30
Whole maize	0.68	3	104
Crushed maize	0.53	13	18

the facts in this experiment and in similar experiments on wheat and maize. This suggests that the self-heating of damp soya beans above the maximum temperature of microbiological activity is due to oxidation of the oil they contain, aided by such mechanical breakdown of the beans as the biological agencies may have caused. From the results in Table 5 it seems likely that wheat would not heat from the same cause. None of the wet wool which heated to 90–100°C in the present experiments contained sufficient unsaturated fats for similar chemistry to be operative, as shown by application of Walker and Williamson's ignition test (1957) to the samples; and, in particular, this is true of the scoured wool wetted with borax buffer described in Experiment 6 above. Moreover, the pie wool (Figs. 1 and 7) which did contain oxidisable fat, failed to heat above 75°C both in bales and in the adiabatic apparatus. It therefore appears that, to explain the self-heating of wet wool to temperatures above, say, 80°C, a different chemical reaction must be postulated to that operative with soya beans.

The results described in Experiment 5 above show that it is not necessary for wool to be saturated with water for self-heating to occur. Heat can develop with wool in equilibrium with atmospheres of RH below 100%. This type of behaviour has been observed in other organic materials; for example, seed grains (e.g., Sallans *et al.* 1944;

Milner *et al.* 1947). These investigators concluded that heating under these conditions was caused by mould metabolism, and pointed out that the more xerophytic moulds can grow at relative humidities down to 74–75%. Indeed Snow *et al.* (1944) have shown that, after prolonged induction periods, active mould growth can take place at humidities below 75%. The first experiment described under Experiment 5 demonstrates self-heating of slipe wool in the adiabatic apparatus at 81% RH, the same humidity as was measured in the bale of this same wool found heated in commerce. It is likely that moulds were responsible for heating both in this bale and in the adiabatic apparatus. The second experiment described under Experiment 5 demonstrates heating in a bale of slipe wool with a moisture content of 38.5%. This would be sufficient to saturate the internal atmosphere within the wool bale. However, Race (1946) has stated that experiments show that degradation of wool is effected almost solely by moulds at regains up to 50%, even when inoculated with both moulds and bacteria. It is generally recognised that the maximum temperature at which moulds can metabolise is about 55°c (Carlyle and Norman 1941; Milner *et al.* 1947). It is therefore likely that this bale was heated by mould attack, although probably by different moulds from those active at 81% RH (cf. Milner *et al.* 1947). When a sample of wool from this bale was conditioned to equilibrium with an atmosphere at 76% RH and maintained in this atmosphere for 80 days in the adiabatic apparatus, it showed no signs of heating. However, when the same sample (without removal from the apparatus) was conditioned to equilibrium with an atmosphere of 81% RH, it heated to 46.7°c. These differences in the rate of heat generation between equilibrium atmospheres of 76% and 81% demonstrate the narrow variation in moisture content that is sufficient to control heating, and are in accordance with published descriptions of fungal metabolism (Galloway 1935; Snow *et al.* 1944). It is possible that experiments with other equilibrium humidities would lead to higher temperatures (cf. Milner *et al.* 1947).

All these experiments offer explanations of the self-heating of wool bales in commerce which are wet for various reasons—for example, sheep shorn when wet. Some bales become accidentally wetted by falling into water, or by rising flood waters. According to numerous trade descriptions, these become very hot indeed, and the causative agents are probably bacteria. Other bales are packed with a moisture content slightly higher than normal equilibrium regain, and these probably heat by mould activity. Galloway and Burgess (1946) have stated that moulds can develop on commercially clean botany wool at 94% RH (corresponding to a regain of 24%), and that mould growth might be established at lower moisture contents in raw wool containing the normal impurities. Burgess (1954) has suggested that the existing commercial standards of regain for wool might be sufficiently high to permit mould attack in some grades of wool, and that it would therefore be advantageous for the textile industry to adopt lower standards of regain to avoid mould growth. The present experiments show that such mould attack could be responsible for heating in baled wool.

The self-heating of wet wool has often been considered as a potential source of spontaneous ignition, probably from analogy with fires in wet hay. Walker and Williamson (1957) described a bale of wet slipe wool which reached a temperature of 70.2°C, but concluded that such heating, of itself, is unlikely to lead to fire. The present experiments demonstrate self-generated temperatures in wet wool under well aerated and reasonably adiabatic conditions of up to 101.8°C. Rises of this type were produced in wool that had a negligible tendency towards spontaneous ignition when dry (as shown by Walker and Williamson's test 1957). However, even when this hot wet wool was exposed to dry air, it showed no tendency to heat above 102°C. When a sample of wet self-heated slipe wool was dried under CO_2 and then exposed to oxygen it failed to generate heat (Walker and Williamson 1957; see also the present Experiment 2). This heating reaction thus appears to be limited to wet wool. Further, no case is yet known of wet wool in a bale heating above 76°C. This temperature has been recorded only in wool over, say, 60% regain, and bales containing less water usually reach lower peak temperatures (e.g., N.Z. Royal Commission 1907; Walker and Williamson 1957). Thus, in spite of the temperatures generated in the present laboratory experiments, it must be concluded that wool that initially has no inherent tendency towards spontaneous ignition (Walker and Williamson 1957) will not present any hazard of ignition when undergoing self-heating through being wet. There are two conceivable reservations to this statement. Some bales of wool contain an admixture of seeds and vegetable matter. It is known that haystacks found heating above 65°C are liable to spontaneous ignition (Dodd 1933), although the mechanism is not known. It is conceivable that bales of wet wool with a high seed content might heat to 70–75°C and then initiate the same reaction in this vegetable matter as occurs in wet hay, and so ignite. This reaction is not considered likely, but in view of the lack of understanding of haystack fires, an open mind must be preserved. Similarly, other bales in commerce contain an admixture of faecal matter ("dags"). An open mind is again necessary, in view of reported spontaneous ignition in stable manure (James *et al.* 1928). Walker and Williamson (1957) tested many samples of both these grades of wool in a dry state, but found no tendency towards spontaneous ignition.

It remains to consider the relation between the self-heating of wet wool demonstrated in the present experiments and the spontaneous ignition of pie wool described by Walker and Williamson (1957). Walker and Harrison (1960) measured the temperature coefficient of thermal output of a single sample of pie wool in air, and showed that the reaction rate doubles for each rise of about 9°C over the range of 45°C to 100°C. Walker and Harrison (in press) showed that other pie wools have similar temperature coefficients for rate of absorption of oxygen at about 70°C, but that there appears to be an anomaly in the temperature coefficient of the heating reaction between 63°C and room temperature. They suggested that this anomaly might occur at the melting point of the fat (pie fats are normally solid at room temperature), since reaction rates at room temperatures seem to be much smaller than would be expected if the measured temperature co-

efficients applied over the whole temperature interval. Carrie *et al.* (1959) experimented with a sample of pie wool that would fire in Walker and Williamson's test (1957) in 66 min., and calculated that a singly dumped commercial bale of perfectly dry wool of this type would not ignite spontaneously in ambient atmospheres up to 40°C. Their calculated temperature distribution shows that such a bale would approach thermal instability (i.e., spontaneous ignition) at temperatures not greatly in excess of 40°C if provided with an adequate internal ventilation. Walker and Williamson (1957) describe many other samples of pie wool that fired in their test in times shorter than 66 min. The practical significance of these facts is that, in commerce involving unscoured pie wool, there must exist many bales of dry wool which are relatively inactive at room temperature, but which could develop appreciable self-heating due to chemical oxidation of the fat if the temperature were raised, say, to 45°C or above, and if adequate internal ventilation were available. If these bales were for any reason wet or damp, the present experiments demonstrate that microbiological heating could raise the temperature to at least 40–45°C.

Walker and Harrison (in press) showed that oxidation of the fat in pie wool can take place in the presence of considerable moisture, although variations in tolerance to water appear to exist amongst various samples of pie wool. These investigators did not employ humidities higher than 50%, since their experiments were performed at temperatures of 100°C and above. The present experiments and those of Walker and Williamson (1957) on bales of wet slipe wool show that, for wet wool to reach temperatures of, say, 70°C or higher, it must have water present in large excess over that necessary to cause saturation of the interstitial atmosphere. Walker and Harrison's results (in press) show that this level of moisture content would greatly reduce the oxidation of pie wool, especially at higher temperatures. For a bale of such wool to rise above 100°C it would be necessary to distill off all water in excess of the 30% required to produce saturation in the air providing internal ventilation. Walker and Harrison's quoted figures (1960) show that the pie wool they experimented with would require a minimum ventilation rate of 3×10^{-9} ml (s.t.p.) dry air/sec/g (dry wool) to support oxidation at 100°C at a rate approaching their demonstrated rate. Simple calculation shows that, if this air were vented at 80°C, a rate of heat generation of 500×10^{-6} cal/sec/g (dry wool) would suffice to dry wool completely from an initial regain of 100% in 13 days. Equation (1) shows that, if a mean thermal conductivity be assumed of 10×10^{-4} cal/sec/sq cm/cm/°C, a rate of heat generation of $1{,}000 \times 10^{-6}$ cal/sec/g (dry wool) would be necessary merely to support a stable central temperature of 100°C in a commercial-sized bale of wet wool. Moreover, since the exposed surfaces would be cool, air could not be vented from the bale at 80°C. At a total heat output of, say, $2{,}000 \times 10^{-6}$ cal/sec/g (dry wool) there would thus be a minimum period of about 10 days spent at 100°C before the bale temperature could rise further.

The present experiments with wet baled pie wool (Experiment 1) show that less than eight days at temperatures around 70°C were sufficient to render the fat in pie wool greatly reduced in its reactivity

with oxygen (Table 1). This change in the nature of the fat may be due to aerial oxidation at these temperatures, or it may be due to bacterial attack. Walker and Harrison (in press) demonstrated that the presence of moisture decreases the rate of reaction of pie wool with oxygen, as also does a reduction in oxygen partial pressure. Experiment 2 described above shows that oxygen partial pressure is considerably reduced in the interior of a wet bale of wool undergoing bacterial heating. These two effects would reduce the rate of simple chemical oxidation of unsaturated fat that could be expected to occur in the central region of the wet bale. Further, the reaction of pie wool with oxygen characteristically doubles for a temperature rise of about 9°c (Walker and Harrison 1960, in press), and yet an experiment (Walker and Harrison in press) showed that maintaining a sample of dry pie wool in air at 60°c for 16 days had a lesser effect on the ignition behaviour (Walker and Williamson 1957) than the effects shown in Table 1. In view of the presence of water and the lowered partial pressure of oxygen in wet heated bales, this suggests that the effects shown in Table 1 are more severe than can be explained by the chemical oxidation that could have taken place, and that the nature of the unsaturated fat may therefore have been modified by bacterial attack. Moreover, the central bale temperatures in Experiment 1 at no stage rose above 73°c, and yet the calculations presented by Carrie *et al.* (1959) suggest that, if aerial oxidation of the fat were occurring to any extent, it would make a large contribution to the temperature rises in the two bales. This contribution would of course be lessened by the presence of moisture and the deficiency of oxygen, and also by the effect of moisture on thermal conductivity and specific heat. However, these experimental facts all suggest that chemical oxidation of the unsaturated fats was not occurring at the normal rate in these heated wet bales. In view of all these facts, it is considered highly improbable that a bale of wet pie wool containing more than, say, 50% water (regain) could ever rise to ignition temperatures.

When microbiological heating is due to moulds, bale temperatures could rise to perhaps 45 55°c. This would be sufficient to raise pie wool from room temperature to a temperature higher than the melting point of the fat (Walker and Harrison in press). This heating could arise with an RH in the interstitial atmosphere of about 80–85%, and this would inhibit the oxidation of pie wool, but not to the same extent as would wool completely wet. The water content necessary to cause this heating (about 20–25%) would not greatly affect the thermal conductivity. There is thus a possibility that heating caused by moulds could lead to subsequent oxidation of the fat in pie wool, so continuing the temperature rise indefinitely. In view of the water relations of wool (W.I.R.A. 1955), water would be evaporated continuously from the bale as the temperature rose, and no inflexion of the heating curve could be expected at 100°c. Detailed experiments are planned to explore this possibility.

Conclusions

1. Wet wool (around 100% regain) in bales or large piles will become hot through the metabolic activity of aerobic mesophilic and

thermophilic bacteria. Thermophiles do not require the prior activity of mesophiles. All grades of wool will heat, but rates of heat generation are much lower in scoured wool.

2. The maximum temperatures which normal thermophiles can develop are 75–76°C. However, strains of thermophiles appear to exist capable of heating wool to 79.4°C.

3. When wet wool is held for several days at high temperatures (say, 80°C), it degrades, and a chemical reaction can commence which is capable of further raising the temperature to about 102°C (at atmospheric pressures) under sufficiently adiabatic conditions. This type of heating has not been observed in baled wool.

4. Aerated damp wool (i.e., wool containing water in insufficient amount to saturate the interstitial atmosphere) can also heat. This heating is believed to be due to the metabolic activity of moulds.

5. Wool which of itself has no tendency towards spontaneous ignition when dry would not have an ignition hazard when wet or damp. Exceptions to this statement might possibly exist in wet seedy wool or wet daggy wool.

6. Bales of pie wool which have tendencies towards spontaneous ignition are unlikely to have an appreciable ignition hazard when heating through being wet. If the bales are merely damp they might show an ignition hazard.

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THE EFFECT OF EARLY WEANING UPON THE ENERGETIC EFFICIENCY OF THE REPRODUCTIVE CYCLE OF SOWS AND UPON MANAGEMENT AND MARKETING METHODS

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Summary

The efficiency of feed energy-conversion of 22 sows and litters weaned between 18 and 49 days was studied and compared with that of 19 sows and litters weaned at 56 days.

Both weaning age (x_1) and litter size (x_2) had a significant effect upon the efficiency of feed conversion (z), together accounting for 49% of the variation in z. The derived regression equation was $z = 0.0336x_1 - 0.10x_2 + 3.157 \pm 0.249$. The feed energy requirement/lb of litter gain between 0 and 8 weeks of litters of 10 pigs weaned at 20 days was 30% less than that of litters weaned at 56 days. When an estimated gestation intake which provided only for maintenance and litter growth was added to the sow and litter intake between 0 and 8 weeks, the saving in feed requirements achieved by weaning at 20 days was reduced from 30% to 21%. Litters weaned between 18 and 26 days, and to a lesser extent those weaned between 26 and 33 days, suffered a check in growth rate at weaning. Litters weaned after 33 days suffered no check. On the other hand, when adjustments were made for litter size, each additional day on the age of the litter at weaning reduced the weight at 8 weeks by 1.65 lb.

Under New Zealand conditions early weaning is economically sound as long as the cost/therm of metabolisable energy of the feed mixtures is less than 1.7 times that of feeds normally used for suckling sows and litters.

Where a prescribed daily feed supply must be disposed of, the adoption of a system of 20-day weaning necessitates 1.2 sows and their progeny being kept where 1.0 sows and their progeny were run under a normal suckling and weaning system.

The reduction in sows and litter feed requirements from early weaning results in a greater proportion of the total feed supply of the herd being devoted to fattening pigs, and this could, in many cases, lead to a lowering of the economical marketing weight of commercial pigs.

Introduction

The technique of weaning litters from the sow before they reach eight weeks of age has been widely adopted in a number of countries in an attempt to increase the efficiency of the reproductive phase of pig production. The arguments advanced in support of this method are logical, namely, that the feed consumed by the litter directly from the trough is converted twice as efficiently as that fed through the sow, and that sow weight losses are reduced as the lactation period is shortened.

Data from sow nutrition studies carried out at this Station have shown that the two major factors contributing to the energetic efficiency of the reproductive cycle of sows are the nature and magnitude of sow weight changes and the level of intake of supplementary food by the

litter in relation to the feed intake of the sow (Smith 1960a, b).

The objects of the present study were:

1. To determine the effect of age of weaning upon the efficiency of energy conversion by the sow and litter from birth of the litter to eight weeks of age.
2. To study relative costs of early weaning compared with those of litters normally suckled for the whole eight weeks.
3. To assess the effects of early weaning upon general management, stocking, and marketing.

EXPERIMENTAL PROCEDURE

Materials and Methods

Records from 22 sows and litters weaned between 18 and 49 days after farrowing and 19 sows and litters normally suckled and weaned at 56 days were available for study. The gross data relating to these animals are shown in Table 1.

TABLE 1. DATA ON SOWS AND LITTERS

Treatment	No. Of Sows	Mean Liveweight			No. Of Pigs Suckled	Mean Wt. Per Pig	
		At Farrow	After Farrow	At Weaning		At Birth	At Weaning
Early weaned	22	426	376	359	9.8	2.72	41
Normally suckled	19	400	350	342	9.7	2.61	40

Both groups of sows comprised first, second, and third litter sows, and the breeds represented were Large White, Berkshire, and Large White × Berkshire cross-breds.

The rations fed to the sows consisted of separated milk and barley meal, and the rate of feeding for the 19 normally weaned and 17 of the early weaned sows was 4.5 units/sow and 1 unit/piglet suckled. The remaining five early weaned sows were fed at the rate of 4 units/sow and $\frac{2}{3}$ unit/pig as part of another experiment.

Litter Treatments

All litters were provided with a pre-starter meal at 10 days of age, the composition of which is shown in Table 2. This material was fed to weaned or suckling pigs until three weeks of age. At this stage a mixture of pre-starter meal and pellets (composition shown in Table 2) was fed for one week. From four to seven weeks pellets were fed alone. During the seventh week a mixture of barley meal and pellets was offered, the proportion of pellets being progressively reduced so that barley meal comprised the total concentrate ration during the eighth week. As soon as the normally suckled pigs commenced to drink milk from the sows' trough they were offered separated milk to appetite in their creep, but piglets weaned before five weeks were not fed milk until this age was reached. From five weeks of age milk was fed to the weaned

pigs in slowly increasing quantities, in three feeds/day, starting at the rate of $\frac{1}{2}$ pint/pig/day and rising to one gal in the eighth week.

The early weaned pigs were taken directly from the sow into a Danish piggery, bedded down in shavings, and provided with a heat lamp. Sow and litter weights were recorded weekly.

TABLE 2. THE FEED MIXTURES USED FOR BOTH NORMALLY SUCKLED AND EARLY WEANED LITTERS (Parts/100 lb)

	Pre-Starter Meal	Pellets
Skim milk powder	65.0	25.0
Fish meal	7.5	5.0
Meat meal	7.5	5.0
Maize meal		10.0
Barley meal		37.5
Sugar	15.0	5.0
Tallow	5.0	7.5
Bran		5.0
Mineral mix 8 oz/100 lb		
Penicillin 0.5 g		
Vitamin A 280,000 I.U.		
Vitamin D_3 28,000 I.U.		

Energy Estimates

Estimates of the energy content of the various feed-stuffs used for the sows and litters have been based, as in previous studies (Smith 1959, 1960a), on t.d.n. values which were then converted to kilocalories of metabolisable energy by using the factor 1,814 kcal/lb of t.d.n. (Brody 1945). The energy content of the litters was calculated using the factors 420 kcal/lb liveweight at birth and 1,055 kcal/lb at eight weeks (Berge and Indrebo 1954).

As shown in Table 1, the normally suckled sows on the average lost less weight over lactation than the sows whose litters were early weaned. There are two reasons for this rather unexpected situation: the first -which has already been mentioned—is that five of the early weaned sows were fed on a lower ration during lactation than the normally suckled sows; secondly, the major loss in weight in almost all cases occurred in the first three weeks of lactation, and in many cases sows fed at the rate of $4\frac{1}{2}$ + 1 gained weight during the seventh and eighth weeks. It is therefore quite clear that, when sows are adequately fed, the claim that early weaning reduces weight losses is not always true and is not necessarily a contributing factor to the greater efficiency claimed for the early weaning technique. For this reason all sow intakes used in calculating the feed energy consumption of the sows and litters/lb of litter gain have been adjusted to "energy intake equivalents" by adding 3,312 kcal/lb of sow weight loss to the observed intakes or by subtracting 4,925 kcal/lb of weight gain (Smith 1959).

The "energy intake equivalent" calculated in this way is the estimated intake necessary to maintain the sow at constant weight throughout lactation.

The other estimates of energetic efficiency used in this paper have been described in a previous publication (Smith 1960a).

The inclusion of the feed energy intakes of sows for the period of gestation, to provide a total feed consumption for the whole reproductive cycle, posed some problems. Since the aim of this study was to determine the effect of age at weaning upon the efficiency of energy conversion, and since the age at which weaning took place was in no way related to, or dependent upon, the level of nutrition during gestation, it was decided to use an estimated feed energy intake which would apply to all sows, both in the early weaned and normally suckled groups. The level of intake over gestation has been shown to have a profound effect upon the energetic efficiency of the reproductive cycle (Smith 1960b) in that sows which gain weight between mating and farrowing provide a greater energy recovery/unit of feed energy invested than those which merely maintained their weight over this period. On the other hand, it has been suggested (Smith 1960b) that the greatest return in terms of carcass weight and money is achieved when the sow is merely maintained during gestation and feed in excess of this requirement is diverted to lactating sows and their weaned and unweaned litters.

For this latter reason it was decided to allow for the maintenance requirement of a 400 lb sow whose weight increased by 50 lb over gestation as a result of the growth of uterine contents; in addition, it was decided to allow for the energy requirement for the growth of a litter of 10 pigs weighing a total of 26 lb at birth. (It will be noted that the average loss of weight over farrowing for both groups of sows included in this study was 50 lb.)

Calculated on this basis, the metabolisable feed energy intake over gestation was 734 therms.

RESULTS

The relevant data for the 22 early weaned sows are shown in Table 3.

These data for the early weaned sows and litters were subjected to a multiple regression analysis, the results of which are summarised below.

Litter Gains (y)

Litter gains were, as expected, highly dependent upon litter size, which accounted for 78% of the variation in gain. When allowance was made for variation in litter size weaning age had a significant effect ($P < .07$) upon gains, accounting for a further 2.6% in the variation, the derived equation being

$$y = 43.8x_2 - 1.65x_1 \pm 33.7 \ (9.0\%)$$

when y = litter gain, x_2 = litter size, and x_1 = age in days at weaning. Each increase in weaning age of one day, therefore, reduced the adjusted litter gains by 1.65 lb.

When litter size was ignored, age at weaning accounted for 18% of the variation in litter gain ($P < .01$).

TABLE 3. THE AGE AT WEANING, NUMBER OF PIGS WEANED, THE SOW AND LITTER ENERGY INTAKE, AND THE LITTER WEIGHT GAINS OF THE EARLY AND NORMALLY WEANED GROUPS

	Age At Weaning (Days)	No. Of Pigs Weaned	Sow Intake Equivalent (Therms)	Litter Intake (Therms)	Litter Weight Gain (lb)	Therms Intake/lb Gain
	18	10	367	756	403	2.79
	21	12	474	720	454	2.63
	21	9	356	698	362	2.91
	23	9	593	653	357	3.49
	24	10	430	685	393	2.84
	24	12	552	1,077	523	3.11
	25	11	507	790	456	2.84
	26	11	435	781	409	2.97
	31	12	521	756	478	2.67
	30	10	594	568	407	2.85
	30	10	534	826	434	3.13
	33	9	530	498	320	3.21
	35	7	538	281	234	3.50
	36	8	629	343	270	3.60
	36	11	683	746	460	3.11
	37	9	646	396	263	3.96
	40	9	701	412	354	3.14
	43	10	785	556	352	3.81
	43	7	628	480	287	3.86
	46	10	939	581	385	3.95
	47	10	874	274	311	3.69
	49	10	967	456	363	3.92
Means	32.6	9.8	603.8	606.0	376.1	3.27
Mean for 19 normally weaned sows	56	9.7	1,043	413	365	3.98

Sow Intake (W_1)

The age of the litter at weaning was found to account for 80% ($P < .01$) of the variation in sow intake. After allowing for weaning age, litter size had a significant effect ($P < .05$), accounting for 4.3% of the variation in W_1. The derived equation was

$$W_1 = 15.7x_1 + 9.5x_2 \pm 70.12\ (11.6\%).$$

Litter Intake (W_2)

Weaning age and litter size respectively accounted for 41% and 48% of the variation in litter intake. Jointly they accounted for 68% of the variance and were both highly significant. The derived equation for litter intake was

$$W_2 = -9.07x_1 + 91.7x_2 \pm 112.0\ (18.5\%).$$

The Combined Intake of the Sow and Litter (W)

Although this combined intake was not significantly affected by weaning age when litter size was ignored, it was dependent to a highly significant degree upon litter size, which accounted for 43% of the variation. However, when allowance was made for litter size, weaning age accounted for a further 10% of the variability ($P < .01$), the derived

equation being

$$W = 101.2x_2 + 6.65x_1 \pm 127.7\ (10.6\%),$$

which indicated that an increase in sow intake was only partly compensated for by a decrease in litter intake.

Efficiency of Feed Energy Conversion by the Sow and Litter ($w = z$)

Both weaning age and litter size had a significant effect upon efficiency, accounting respectively for 62% and 26% of the variation. Together these two factors accounted for 69% of the variation in z. The effect of litter size was significant ($P < 0.5$) when allowance was made for weaning age.

The derived equation was

$$Z = 0.0336x_1 - 0.10x_2 + 3.157 \pm 0.249\ (7.6\%).$$

It is apparent that the earlier a litter is weaned the greater is the efficiency of feed conversion.

The above data were derived from litters whose conditions of housing and feeding were, as far as present knowledge allows, ideal. Despite this fact, litters weaned between 18 and 26 days, and, to a lesser extent, those weaned between 28 and 33 days, suffered a setback at weaning.

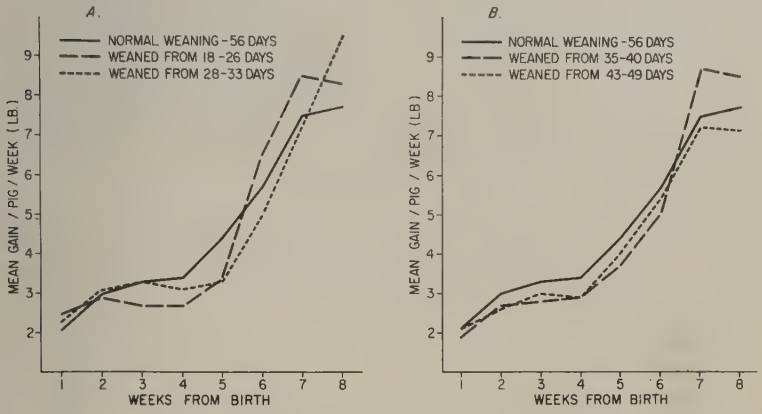
A.
NORMAL WEANING - 56 DAYS
WEANED FROM 18 - 26 DAYS
WEANED FROM 28 - 33 DAYS
MEAN GAIN / PIG / WEEK (LB.)
WEEKS FROM BIRTH
B.
NORMAL WEANING - 56 DAYS
WEANED FROM 35 - 40 DAYS
WEANED FROM 43 - 49 DAYS
MEAN GAIN / PIG / WEEK (LB.)
WEEKS FROM BIRTH

Fig. 1.—The mean weekly weight gains per pig from litters weaned at various ages.

It is probable that, under anything less than ideal conditions, an accentuation would occur in the reduction in growth rate shown in Fig. 1 for litters weaned prior to 28 days; this view is supported by results achieved on farms. It is during the period immediately following weaning, when the piglets are adjusting to the absence of the sow, that intestinal upsets occur among poorly managed litters, resulting in static weights and low efficiency for a considerable period thereafter. Under such circumstances the conclusion reached above, that efficiency increases as the age of weaning is lowered, would not necessarily hold true for litters weaned into poor conditions before four weeks of age.

Under the conditions and management provided, however, when allowance was made for litter size, the earlier a litter was weaned the heavier it was at eight weeks—despite the setback at weaning—and the higher the efficiency of feed energy conversion; the feed energy requirement/lb of gain fell from 4 therms for litters of 10 weaned at eight weeks to 2.8 therms for those weaned at 20 days—a 30% reduction. The mean energy requirement/lb of gain of the 19 normally suckled sows not included in the analysis was 4.09 therms.

The Effect of Early Weaning upon the Efficiency of the Reproductive Cycle

When the estimated feed energy intake over gestation was added to the observed lactation intake of the sows and litters the energy requirement for the whole reproductive cycle/lb of litter gain was raised from 2.8 therms for litters of 10 pigs weaned at 20 days to 4.4 therms, and from 4.0 at 56 days to 5.6 therms. Thus the inclusion of a minimum gestation intake of 734 therms reduced the percentage increase in feed conversion efficiency due to weaning at 20, rather than 56, days from 30% over lactation alone to 21% over the whole reproductive cycle.

The Efficiency of Energy Recovery

In previous studies in this series the criterion used for the efficiency of the lactational and gestational phases of the reproductive cycle, and for the whole cycle, has been the energy recovered in the form of sow and litter tissue for each 100 therms of the observed intake. In the present study sow intakes were adjusted as described earlier on an "energy intake equivalent" basis, so that sow weight changes could be ignored, and this same procedure was used in calculating the efficiency of energy recovery. The values obtained for the whole reproductive cycle were submitted to regression analysis, the derived equation being

$$y = 25.94 - 0.124x_1$$

where y = energy recovery in the form of litter tissue for 100 therms of sow and litter intake, and x_1 the age in days at weaning. The estimated energy recovery for litters weaned at 56 days was 18% and for litters weaned at 20 days 23.5%.

Discussion

Many advantages have been claimed for the early weaning technique, namely, that: (i) more litters can be obtained from the sow in 12 months; (ii) excessive sow weight losses can be more easily avoided; and (iii) efficiency of feed conversion is improved.

The present study is a somewhat formalised attempt to establish the order of the feed savings which might reasonably be expected from the technique when applied at various ages. It was felt that, in a study of this type, no regard should be taken of fortuitous sow weight losses, and, in fact, previous work has indicated that, in the interests of efficiency, these losses should not be allowed to occur. The formalisation was confined to this aspect of the problem.

Three facts emerge from the results presented:—

1. When lactation efficiency alone is being considered the feed energy required/lb of litter gain, for litters of 10 pigs, can be reduced by

as much as 30% as a result of weaning at 20 days instead of at the traditional 56 days.

2. When the minimum energy requirements for gestation are added to those for lactation the saving in feed energy/lb of gain is reduced from 30% to 21%.

3. The energy recovery in the form of litter tissue/100 therms of feed energy consumed is increased from 18 therms for litters weaned at 56 days to 23.5 therms for those weaned at 20 days.

These findings indicate the energetic efficiency of the early weaning system, but they do not indicate the economic advantages, or the limitations, of the system.

The development of early weaning as a practical method of rearing litters has awaited, and followed, the evolution of satisfactory baby pig rations. These have been developed to a stage where many recipes are available for use. The majority, if not all, include such a large number of feed ingredients and additives that the farmer prefers to purchase his requirements from commercial firms. These commercial preparations are relatively expensive, so that an estimate of the price that can be paid for early weaning feedstuffs relative to that paid for concentrates at present being used by New Zealand farmers merits a place in this discussion.

After application of the equations developed earlier—sow intake $W_1 = 15.7x_1 + 9.5x_2$ and litter intake $W_2 = -9.07x_1 + 91.7x_2$—the following values were obtained for the relative intakes of sows and litters weaned at various stages of lactation:

TABLE 4. THE ESTIMATED INTAKES OF SOWS AND OF LITTERS WEANED AT VARIOUS AGES

Age At Weaning (Days)	Sow Intake (Therms)	Litter Intake (Therms)	Total
20	409	736	1,145
30	565	645	1,210
40	723	554	1,277
50	880	464	1,344
56	974	409	1,383

The combined intake of the early weaned sows and litters was less than that of those normally suckled, but, in addition, the early weaned litters were heavier at eight weeks. The estimated weight gain of litters of 10 pigs weaned at 20 days of age was 59 lb greater than the gain of litters of similar size weaned at 56 days ($y = 43.8x_2 - 1.65x_1$). This extra gain was estimated to require 170 therms of feed energy. To provide a comparison with normally weaned litters, on the basis of similar litter gains, this 170 therms may be subtracted from the 409 therms consumed by sows weaned at 20 days; the estimated sow intake is thus reduced to 239 therms which comprised separated milk and barley meal.

The litter intake of 736 therms, when apportioned on the basis of the mean percentage intake of each feed actually consumed, consisted

of 191 therms in the form of milk and barley meal and 545 therms in the form of pre-starter meal and pellets.

The total energy consumption in the form of barley meal and milk by the sow and litter was thus 430 therms. If it is assumed that the normally suckled litters were fed on the cheapest concentrates available, that is, barley and meat meal, as was the practice at this Station prior to these present experiments, the total saving in milk, barley, and meat meal would have been 953 (i.e. 1,383 — 430) therms of feed energy. To achieve this the early weaned litters consumed 545 therms in the form of pre-starter meal and pellets. On an equal return basis, therefore, the price/therm of metabolisable energy for an early weaning feed mixture should not be greater than 1.7 times the price/therm of barley or meat meal; or, on the basis of the energy content of the early weaning mixtures used in these experiments, the price/lb should not be more than twice that of barley and meat meal.

Since dairy by-products constitute the bulk of the feed used for pigs in New Zealand, and since these are produced by the dairy herd irrespective of the feed requirements of the pig herd and are perishable, any feed saved by the introduction of more efficient production methods must

TABLE 5. THE EFFECT OF EARLY WEANING UPON COMBINED SOW AND LITTER FEED ENERGY CONVERSION AND THE DISTRIBUTION OF FEED BETWEEN REPRODUCTION AND FATTENING

(a) Feed Energy Requirements of Sows and Fattening Pigs, Calculated from Offered Feed (Grass Excluded)

Type Of Production	Light Pork				Bacon			
	Reproductive Cycle		Growth 40–100 lb		Reproductive Cycle		Growth 40–190 lb	
	Therms Feed lb/ Gain[1]	% Of Total Feed	Therms Feed lb/ Gain[2]	% Of Total Feed	Therms Feed lb/ Gain	% Of Total Feed	Therms Feel lb/ Gain[3]	% Of Total Feed
Treatment: weaned at 8 weeks	4.6	44	3.3	56	4.6	20	4.6	80
weaned at 20 days	3.3	40	3.3	60	3.3	16	4.6	84

(b) Feed Energy Requirements Calculated on Total Feed Energy Consumption

Treatment: weaned at 8 weeks	5.6	52	3.3	48	5.6	24	4.6	76
weaned at 20 days	4.4	49	3.3	51	4.4	20	4.6	80

[1] All calculations based on litters of 10 suckled and fattened.
[2] Based on a feed conversion of 2.5 lb of meal/lb of gain.
[3] Based on feed conversion of 3.5 lb of meal/lb of gain.

be used at the time the saving is made, and this can only be done if extra stock is provided for this purpose.

This increase in stocking will comprise additions to the sow herd and their progeny in the fattening styes, and the extent of these additions has been calculated below.

Under New Zealand production conditions the feed requirement of the pregnant sow can be met by the provision of 200 therms of feed energy in the form of concentrates or milk, the remaining 530 therms being provided from pasture. Milk and concentrates to the value of 609 therms are, therefore, required during the reproductive cycle of a sow weaned at 20 days, and 191 therms from the same source for the litter. A litter of 10 pigs requires approximately 2,000 therms of feed energy to grow from 40 to 100 lb liveweight, making a total feed requirement in the form of milk and concentrates of 2,800 therms compared with 3,580 therms for normally weaned litters. On the basis of these calculations, where weaning at 20 days is instituted, 1.2 sows and their progeny could be kept where 1.0 was previously kept under a system of normal weaning at eight weeks.

Further effects of early weaning, both under New Zealand conditions and under conditions prevailing in countries in which pasture is not so intensively exploited, are shown in Table 5.

The main points illustrated in Table 5 are as follows:

1. As the efficiency of energy conversion over the reproductive cycle is raised by early weaning, the proportion of the total energy intake of the herd devoted to fattening is increased.

2. The effect of early weaning was to increase the energy conversion efficiency of the reproductive cycle above that of the fattening phase of pigs marketed at 190 lb; this was the case both under New Zealand conditions, where the values were related only to milk and concentrate utilisation, and under the more general conditions prevailing in other countries, for which the total energy intake was considered. The only method of bringing these values closer is to market the fattening pigs at a lower weight. This is an illustration of the principle that, as sow overhead feed costs are reduced, the economic marketing weight of fattening pigs is lowered (Atkinson and Klein 1945; Smith 1951).

It is appropriate here to try to assess the ultimate efficiency which is likely to be achieved by weaning at the earliest possible age. Perrin (1955) has shown that the change from colostrum to normal milk takes place over the first four days after farrowing. At the present stage of knowledge in regard to the development of antibodies in young animals, it is reasonable to suggest that four days is, therefore, the minimum practical age for weaning. The estimated efficiency of litters weaned at this age, based on the present data, would be 2.03 therms/lb of gain. Previous work has indicated that the energy intake of sows' milk/lb of litter gain varies between 2.1 and 2.5 therms. It may, however, be possible to reach levels as low as 2.0 therms/lb of gain under early weaning conditions where competition for teats and variation in production among glands no longer have any limiting effect upon growth. The limiting factor in the achievement of such high efficiency appears to

lie in the development of pre-starters, which are palatable to the young pig at weaning, and in the development of techniques which will ensure a high level of intake by the piglets within the first 12 hours after their removal from the sow.

Acknowledgments

Thanks are due to Dr A. H. Carter for the statistical analysis of the data presented.

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PHOTOSENSITIVITY DISEASES IN NEW ZEALAND

XXIII. ISOLATION OF SPORIDESMIN, A SUBSTANCE CAUSING LESIONS CHARACTERISTIC OF FACIAL ECZEMA, FROM *Sporidesmium bakeri*, SYD.

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(*Received for publication, 25 August 1960*)

Summary

Further concentration of the poison from dried pasture was achieved.

Concentration of the poison from wet cultures and spores of *Sporidesmium bakeri* Syd. has resulted from application and modification of methods used for pasture.

Application of two steps using partition-chromatographic columns has allowed isolation of an apparently pure substance, named sporidesmin, from wet cultures and spores.

Sporidesmin causes liver damage in guinea-pigs that is indistinguishable from that produced by wet cultures or spores of *Sporidesmium* or by toxic pasture.

Sporidesmin is resinous, but gives a beautifully crystalline adduct with carbon tetrachloride.

A further crystalline substance, m.p. 244°C, has been isolated from the fungus. It seems non-poisonous.

Introduction

The preparation of concentrates of a liver-damaging poison from facial eczema toxic pasture has been reported by White (1958a), and preliminary evidence on the chemistry of other compounds from toxic grass has been given by White (1958b).

The history of the discovery of the fungus *Pithomyces chartarum* (Berk. & Curt.) M. B. Ellis (Syn. *Sporidesmium bakeri*, Syd.) as the source of the "beaker test" substance and of a liver-damaging poison is covered by Percival and Thornton (1958), Thornton and Percival (1959), Percival (1959a, b), and Thornton and Ross (1959). Progress made in concentrating the poison from cultures of this fungus was reported briefly by White (1959).

A summary of an isolation procedure for a liver-damaging poison named sporidesmin, and a preliminary description, were published by Synge and White (1959). The present paper records in more detail methods for isolation of sporidesmin.

Experimental

Further toxic pasture samples were used, obtained as described previously. Cultures of *Sporidesmium* were mainly those grown in Roux bottles on a potato-carrot medium at 25°C for 7 days, and more than

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40 weekly batches of 100 bottles each were used. Spores grown in different ways were supplied by Drs T. Vernon, P. J. Brook, and R. H. Thornton, the latter using the rye-corn method (Lloyd and Clarke 1959). Later a batch of 7 lb of spores was produced at Ruakura using the rye-corn method, and wet-harvested.

Feeding techniques for guinea-pigs have been described (White 1958a). Liquid cultures or aqueous fractions were deep-frozen in plastic bottles and used in place of water for mixing with grass and milk powder. Spores were dispersed in the milk powder for feeding.

At a later stage of the work, involving the partition columns, the HeLa cell technique investigated by Murphy and Worker (1960) was developed by Dr J. Done*, and a rabbit-eye test was found by Mr P. Mortimer*. Both these tests gave assessment of toxicity levels of extracts of the same order as those obtained from guinea-pig feeding, and speeded up the work appreciably at the end. The chromatographic method for estimation of sporidesmin (Russell 1960) was available some time after the isolation of sporidesmin, and has been of value in the later stages of the spore work.

In terms of standard guinea-pig feeding to produce moderately severe liver damage, the levels of toxicity of starting materials suitable for processing are as follows: 1–2 lb dried toxic pasture, ½–1 Roux bottle (200 ml) of potato-carrot culture, or 100–200 mg of spores. Sources of lower toxicity gave more severe losses on processing, and were not worth the effort.

Preparation of Solvents and Packings for Partition Columns

Carbon disulphide of technical grade was purified by the method given by Vogel (1948); for the Analar grade each treatment was reduced in number and time.

Other solvents used were Analar grade, redistilled to diminish non-volatile residue. All compositions of solvent systems are given by volume.

A quantity of 450 g of Hyflo-Super-Cel (Johns-Manville) was stirred with 3N HCl for 1 hour, filtered on a large Buchner funnel and washed with 3N HCl until no more yellow was removed; the Hyflo was then washed with water, 500 g of 90% (W/V) phenol, 1 L. of 1 : 1 (V/V) benzene-methanol, and finally with 2 L. of methanol, then dried at 125°C.

Purified Hyflo was rendered hydrophobic with dichlorodimethylsilane in a desiccator, following Howard and Martin (1950). It was washed extensively, after being packed in a tube, with methanol, chloroform and benzene, and with the bottom phase of the CS_2/methanol/water system, and dried at 105°C.

Isolation of Sporidesmin

Isolation from Wet Cultures of Sporidesmium

The sources for most of the work on systematic isolation of the poison were cultures prepared on a weekly basis of 100 Roux bottles.

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Steps of isolation (1–5) are described in terms of 100 bottles (18 L.) of culture:

1. The culture is homogenised in a top-drive macerator and shaken out in suitable portions with four equal volumes of de-peroxided ether. The ether is blown off in shallow dishes in a fume duct and the residues collected into a small volume of ether. The aqueous layer containing only a trace of poison is discarded. The ether is blown off and the product dried in a vacuum desiccator.

   An alternative is to basket-centrifuge the culture without homogenising. The felt is suspended in about 1 L. of water, homogenised and extracted with ether as above, and the aqueous part is extracted with one equal volume of ether.

2. The dried extract is taken up in small volumes of ether, totalling about 50 ml, and insoluble residue (largely "beaker test" substance) filtered off on a No. 2 sinter. The soluble part is evaporated to dryness.

3. The residue is taken up in methanol, totalling about 70 ml, any insoluble material filtered off, the solution diluted to 80% by addition of water, and extracted 5 times with equal volumes of petroleum ether (b.p. 40°–70°C).

4. The methanol phase is concentrated, and the poison transferred to ether, and dried as described in Step 6 (White 1958a).

5. The residue is dissolved in a minimum of ether, 5 mg of carotene added, subdivided and chromatographed in ether on three 10 in. × 1 in. alumina columns prepared as described in Step 7 (White 1958a), with the modification that 600 ml ether is used for the preliminary washing of each column.

6. Operations from here on are done in a cold room at 0 — + 2°C in subdued light.

   Eighteen g of purified Hyflo-Super-Cel are mixed with 12 ml of the methanol-rich phase of CS_2/methanol/water, (100:80:20), equilibrated at 0 — + 2°C. This Hyflo is slurried with the other phase into a one-inch column and packed with a packing tool. A column 10.7 – 11.9 cm long results. Evenness of packing is checked by running through a little carotene in the CS_2 phase. The residue from Step 5, coming from 250 bottles of culture, is dissolved in a minimum of the CS_2 phase, put on the column usually with a few drops of the other phase, to facilitate solution, and a head of at least 25 cm of CS_2 phase added. Fractions are collected in units corresponding to 1 cm descent of the liquid surface above the column and measured from the first emergence of colour.

   The poison normally occurs in fractions 11 – 22, and, as an aid to location, 5 µl portions of fractions in this region are put on a paper chromatogram (disc method, Whatman No. 1 paper–White 1957), developed with water for 2 hours, and the poison is located at an R_F (circ.) value of 0.51 by staining with azide

iodine reagent (Russell 1960) or with 0.05% aqueous permanganate. Fractions showing this spot are evaporated under filter-pump vacuum in the cold room, and bulked using ether.

7. Eighteen g of hydrophobic Hyflo-Super-Cel are mixed with 12 ml of the bottom phase of the system chloroform/benzene/methanol/water (15 : 15 : 23 : 7) (Gregory and Craig 1948) and packed into a 1.0 in. dia. tube using top phase and a packing tool. A column 11 cm long results. The toxic fraction from two lots at Step 6, weighing up to 100 mg, is dissolved in a little top phase and put on the column, which is then covered with top phase. Fractions are collected in 1 cm units, starting measurements from the point of putting on, and continued until 35 fractions are collected.

   Fractions 1–12 are bulked, also the next 5, and the next 15 are evaporated individually.

   The poison usually appears in fractions 17–32 and is located by the sharp peak of weight (which tends to have its leading edge less sharp than its tailing edge) or by paper chromatography as used under (6). Evaporation of toxic fractions is done in the cold room under filter-pump vacuum, using a rotary evaporator for larger volumes, and dried by vacuum desiccation.

8. Toxic fractions from (7) are bulked, apart from one or two at the edges of the peak, and dissolved in a minimum of ether, filtered on a sinter, then carbon tetrachloride is added and the solution allowed to evaporate slowly in the cold room. Later more carbon tetrachloride may be added, and the crystals of crude sporidesmin–CCl_4 complex filtered off.

Isolation from Spores of Sporidesmium

Spores used for isolation work were grown by the rye-corn method and gave moderate to severe liver damage by standard guinea-pig feeding at the 100–200 mg level.

The isolation procedure is modified slightly, due to the necessity of using methanol as the initial extracting solvent. Spores used contained 30–50% moisture and all weights used refer to such spores before drying.

1S. Spores are extracted in about 125 g batches in extractors of the type described by Letham *et al.* (1957) and are securely packed in between cotton wool. A 12-hour extraction with methanol is used.

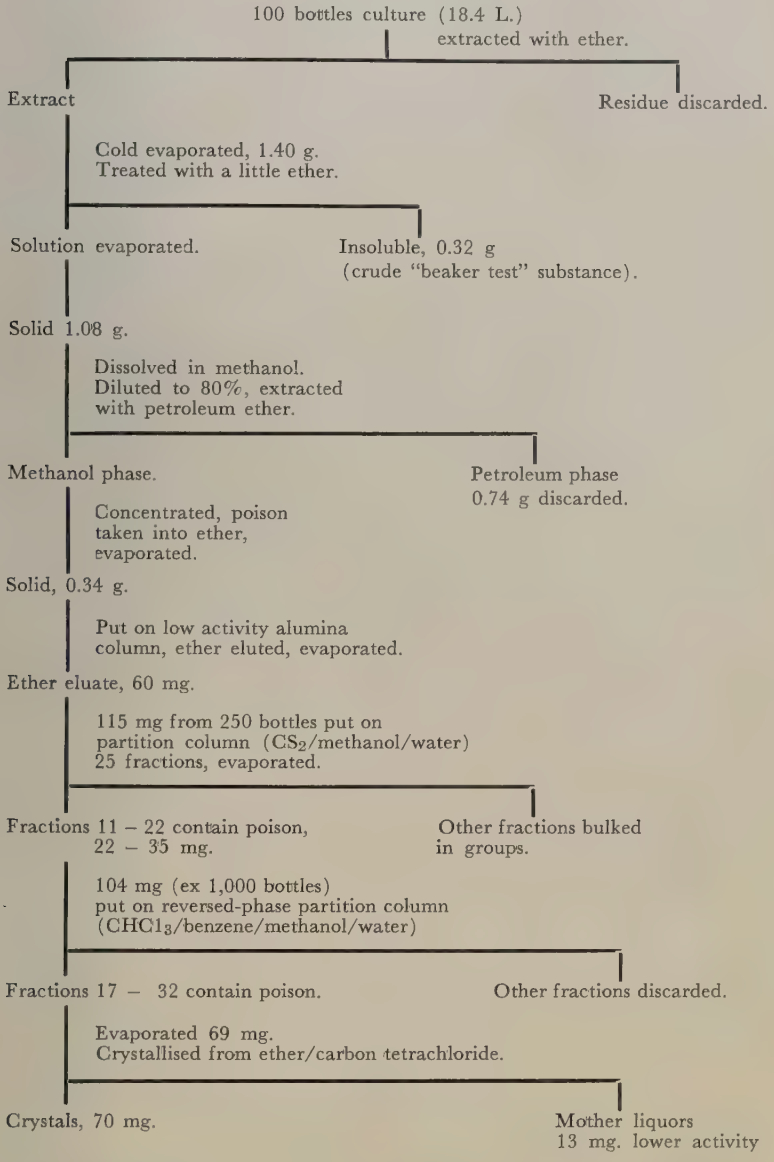
2S. After standing overnight, crude "beaker test" substance is filtered off. The methanol is diluted to 80%, and extracted with petroleum ether.

3S. Methanol is evaporated in the usual way.

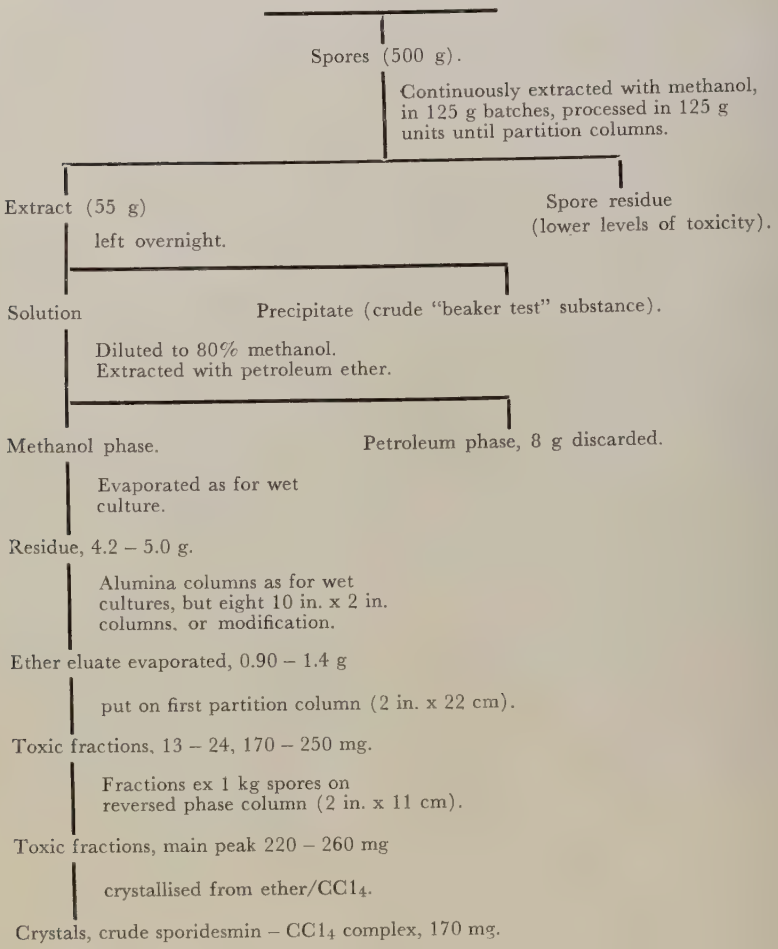
4S. The dried residue is taken up in small volumes of ether, totalling 120 ml, and insoluble residue removed.

5. Two 10 in. × 2 in. alumina columns are used and volumes scaled in proportion. See under "Discussion of Isolation Procedures" for modification.

Scheme of Fractionation and Typical Weight Concentration for Wet Culture



Scheme of Fractionation and Typical Weight Concentrations for Spores


Spores (500 g).
Continuously extracted with methanol, in 125 g batches, processed in 125 g units until partition columns.
Extract (55 g)
Spore residue (lower levels of toxicity).
left overnight.
Solution
Precipitate (crude "beaker test" substance).
Diluted to 80% methanol.
Extracted with petroleum ether.
Methanol phase.
Petroleum phase, 8 g discarded.
Evaporated as for wet culture.
Residue, 4.2 – 5.0 g.
Alumina columns as for wet cultures, but eight 10 in. x 2 in. columns, or modification.
Ether eluate evaporated, 0.90 – 1.4 g
put on first partition column (2 in. x 22 cm).
Toxic fractions, 13 – 24, 170 – 250 mg.
Fractions ex 1 kg spores on reversed phase column (2 in. x 11 cm).
Toxic fractions, main peak 220 – 260 mg
crystallised from ether/CCl_4.
Crystals, crude sporidesmin – CCl_4 complex, 170 mg.

6. The concentrate from 500 g of spores is put on a CS_2/methanol/water partition column 2 in. dia. × 22 cm long, and 2 cm fractions collected. The weight of Hyflo is 144 g (96 ml of stationary phase). The peak of poison is in the same place as for the smaller columns.

7. The reversed-phase column for concentrate from 1 kg of spores is 2 in. dia. × 11 cm long, containing 72 g Hyflo (48 ml of stationary phase). One cm fractions are collected.

8. Crystallisation is as described above.

Discussion of Isolation Procedures

Wet Cultures

1. Aqueous culture residues from several batches were fed after the ether extraction at a level of 200 ml over the feeding period (3 weeks) with no liver damage showing. However, comparative feeding of several batches of the original macerate by Mr J. C. Percival, and feeding of equivalent amounts of dried ether extract, revealed an appreciable loss of poison, apparently occurring in the evaporation stage.

   There is a significant amount of poison in the aqueous liquor when felt is centrifuged off, often half the total (Clare pers. comm.). Appreciable saving of ether results from the basket-centrifuging technique, and from using only one extraction of the aqueous part. Feeding of aqueous phases formed during evaporation of wet ether extracts showed little poison, perhaps 1/50 of the total, in the water.

2–4. Feeding of very high levels of side fractions gave only traces of toxicity.

5. The alumina column, used exactly as described previously (White 1958a), generally showed appreciable loss of poison, about a quarter being recoverel. Subsequent elution with ether containing 1% of methanol sometimes gave traces of poison. Variations in the size and number of columns gave no consistent improvement. Further washing with ether, and the addition of carotene, gave improved recoveries generally, and in many cases feeding work failed to show any significant loss at this stage.

6. Several diameters of columns have been used to suit the weight of concentrate put on, and all weights and volumes have been scaled in proportion. In developing this step 1 cm fractions were collected, evaporated in small flasks, and weighed on a microbalance. The peak of weight about fractions 11–20 was shown to be associated with toxicity, and at this stage it was evident that HeLa cell tests and rabbit-eye tests were capable of giving much the same results as guinea-pigs.

   Fractions were also hydrolysed in a mixture of 0.2 ml 10 N HCl and 0.2 ml acetic acid at 105°c for 24 hours in a sealed evacuated tube; the contents were evaporated and chromatographed on Munktell OB paper using butanol/acetic acid/water (40 : 5 : 50). Ninhydrin revealed the presence of amino acid components in all fractions, the toxic ones being associated with a spot in the position of alanine (also present in some other fractions), and, more specifically, with a spot in the position of glycine. Spots in the leucine and valine positions indicated peptide-like material with partition properties very similar to those of the poison.

   The appearance of a greenish colour (later turning yellow) on heating for the hydrolysis was associated with all toxic fractions. The peak of poison is in a zone corresponding to a

partition coefficient of 4.8 in favour of the stationary phase. When sporidesmin had been isolated and was found to be detectable as a spot of R_F 0.5 (ascending) or 0.51 (circular method—White 1957), using water as developing solvent, this technique was used to detect the beginning and end of the sporidesmin fractions. Staining with aqueous permanganate gave a transient yellow spot, the more sensitive azide-iodine spray a white spot (Russell 1960), the azide stain often detecting sporidesmin in a fraction before (or after) the limits found with permanganate. The peak just before the poison in fractions 5–9 contains a substance detectable with the azide-iodine spray and of R_F 0.21 (circ.). It also contains a substance of m.p. 244°C $[\alpha]_D$—95° (C, 1.33 in acetic acid) which does not stain with azide, and gives one spot only, in the alanine position on hydrolysis: this substance is similar in physical properties to the "beaker test" substance.

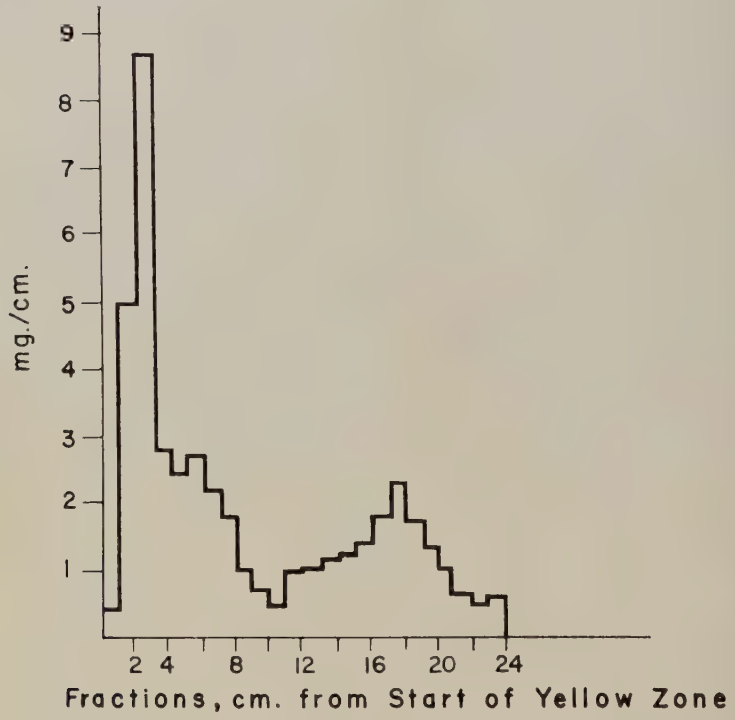


Fig. 1.—CS_2/methanol/water, partition column (10.7 cm × 1 in.), 68.8 mg put on ex 100 bottles culture, PC7 26/27.

A histogram of weight for this system is shown in Fig. 1, and the relations of fractions to toxicity are shown in sections (a) and (b) of Table 1. The system CS_2/methanol/water

TABLE 1. ACTIVITY OF FRACTIONS FROM PARTITION COLUMNS

(a) *Culture concentrate PC7 24/25, CS_2/methanol/water*

Fraction No.	3	5	7	9	11	13	15	17	19	21	23	25
Wt. fraction (mg)	3.11	2.89	3.81	1.83	0.50	0.30	0.76	1.63	1.18	0.41	0.14	0.06
Guinea-pig feeding: mg fed	0.78	0.72	0.95	0.46	0.14	0.074	0.19	0.41	0.29	0.10	0.036	1.44
grading	N	N	N	N	N	N	S	S	S	Mod.	M-Mod	V. Mild†
HeLa cell toxicity*	0.8						0.003	0.009	0.003	0.003	0.003	0.8

(b) *Culture concentrate PC7 26/27, CS_2/methanol/water*

Fraction No.	2	4	6	8	10	12	14	16	18	20	22	24	Wash off with excess stationary phase.
Wt. fraction (mg)	5.03	2.83	2.72	1.85	0.70	1.00	1.07	1.35	2.30	1.33	0.68	0.50	39
Guinea-pig feeding: mg fed	0.63	0.35	0.34	0.23	0.09	0.12	0.13	0.17	0.29	0.17	0.08	0.06	4
grading	N	N	N	N	N	N	S	S	S	M-Mod	N	N	N†
HeLa cell toxicity*	—	—	—	0.4	0.1	0.006	0.003	0.003	0.003	0.003	0.012	0.012	0.12

(c) *Culture concentrate PC7 24/25. Reversed-phase column*

Fraction No.	7–14	15–17	18	19	20	21	22	23	24	25–36	37–115	Wash off with excess stationary phase.
Wt. fraction (mg)	0.51	0.64	0.14	0.29	0.69	1.33	1.80	0.78	0.07	0.26	tr.	1.17
HeLa cell toxicity*	0.4	0.05	—	0.003	—	0.003	—	0.0015	—	0.006	0.4	>0.4

* Least dose (μg of fraction per ml culture) to give a cytotoxic effect, assessed microscopically.

† N, normal; S, severe; Mod., moderate; M-Mod., mild to moderate; V.mild, very mild. Assessment of liver damage according to Perrin (1956).

(100 : 70 : 30) was also used initially, but the peak of poison was closer to the initial peak of weight, and separation was not so well defined.

The fraction obtained by washing the columns with stationary phase gave appreciable weight, but negligible toxicity.

7. The reversed-phase system provides a necessary removal of trace impurities which prevent crystallisation of sporidesmin. Some of these showed "leucine" and "valine" spots on chromatography after hydrolysis. It is advisable to keep separate sporidesmin containing fractions at the sides of the peak, since they do not crystallise easily. The main peak, partition coefficient 6.9 in favour of the stationary phase, consists of sporidesmin.

A histogram of weight is given in Fig. 2, while evidence associating the toxicity with the peak is presented in section (c) of Table 1.

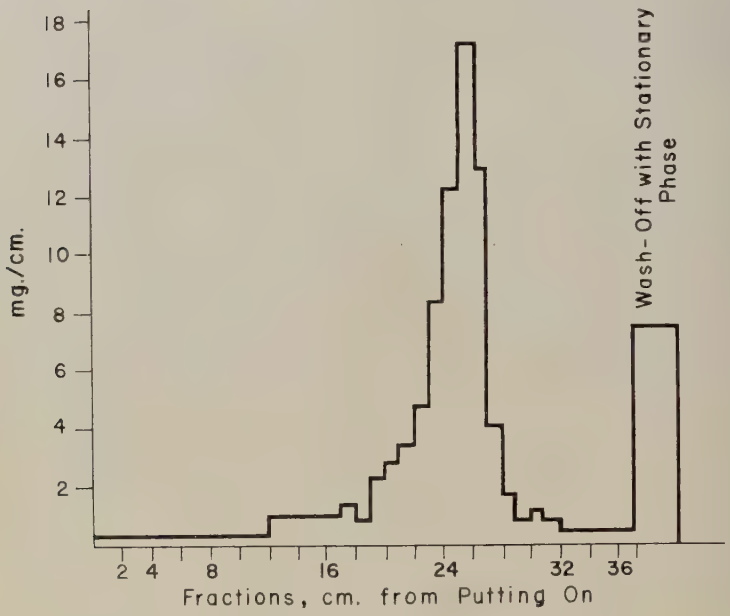


Fig. 2.—Reversed phase column, 104 mg culture concentrates.

Spores

1S. Unground spores produced by various methods were percolated in thin tubes and extracted in various types of small continuous extractors with ether, acetone, and chloroform. Continuous extraction of spores broken up by grinding with carborundum was also done. In all these experiments one-third to two-thirds of the poison could be extracted, the extent of the

extraction appearing to depend on the mode of preparation of the spores. Further extraction with these solvents removed very little of the remaining poison, which could be demonstrated by feeding the residual spores at a higher level.

Spores produced by the rye-corn method were continuously extracted with methanol in small extractors for 14 hours, and one-fifth to one-tenth of the poison was shown to remain in the spores. Large-scale extraction this way showed a similar efficiency of extraction, and the poison was in the methanol without significant loss. The residual spores on re-extraction with methanol for 12 hours were reduced to very low levels of toxicity, and appreciable poison was in the methanol. When this extract was processed, however, much of the poison disappeared.

Shaking for four hours, with cold water or with 0.5% Cetrimide* in quantity far exceeding that required to dissolve the sporidesmin present, removed about 10% of the poison. Grinding of spores (0.5 g) with 10 ml 5% aqueous Tween 40† gave most of the poison in the aqueous phase, from which it could be removed with ether. Worker (1960) found this technique to be efficient for extraction of sporidesmin, using intraportal injection of rabbits. Much higher concentrations of Cetrimide removed more of the poison.

3S. In some runs the methanol phase was evaporated to dryness in the rotary evaporator, and the residue taken up in ether, when about 85% of the weight remained insoluble and the insoluble part usually retained only low levels of poison. The ether solution made this way contained more weight and impurities than that using the preferred method.

4S, 5, 6. With spore extracts there are usually appreciable residues of low solubilities in ether and solvents used. Provided the residues are washed well they contain no more than traces of sporidesmin.

5S. During processing of later batches of spores, the losses of sporidesmin on alumina columns have been reduced greatly by the following modifications. For the concentrate from 250 g of spores a column 5 in. × 1 in. was adequate to give the same weight concentration as the larger columns. The alumina was de-activated by shaking with the proportional amount of acetic acid in ether for 1 hour. It was then packed in the tube and washed with twice the amount of ether previously used. One hundred and fifty ml of eluate measured from the first emergence of carotene was adequate to collect all the sporidesmin.

6. When the partition system was tried on a spore concentrate which had not been put through the alumina column step, poison appeared in the four major fractions collected.

* Cetavlon, I.C.I.

† Polyoxyethylene sorbitan monopalmitate, Atlas Powder Co.

Other Information on Isolation Methods

Grass

With some toxic grass samples the poison did not appear in the normal place on the activity V alumina column with benzene and was not recoverable in any fraction.

After the 70,000 stage, further concentrations were achieved by the following methods. The concentrate was put on another magnesium carbonate column in CS_2 containing 5% ether. Elution with this solvent removed some weight but no poison, but CS_2 containing 0.5% methanol or 25% ether eluted the poison. Concentrations achieved were 150,000–400,000 times, though toxicity was often lost.

The partition system CS_2/methanol/water (100 : 70 : 30) was used with the methanol phase on Celite, silica gel, or cellulose. Elution with the CS_2 phase gave the poison in a fraction behind the solvent front, and concentrations of 500,000–1 million (in one case, 2 million) were obtainable.

Both these techniques gave a few inconsistent results.

Fungus Cultures

Some other techniques beyond the acetic acid/alumina stage gave appreciable concentrations, but were inconsistent.

Alumina V columns were used, concentrates put on in benzene, and elution continued with this solvent, then with small volumes of benzene containing the following percentages of ether: 1, 2.5, 5, 10, 15, and 20%. There was good distribution of weight between the fractions, and sporidesmin usually appeared in the eluates from benzene containing 1.5–5% ether. The nicely crystalline compound m.p. 244°c appeared just in front of the poison. A doubling of concentration resulted from elution, first, with benzene, then with benzene containing 20% ether.

Poison was not usually recoverable from a zinc carbonate column, but in one case acetone elution was successful, after several less polar solvents had failed.

Concentrates were put on columns of Mallinckrodt's "silicic acid" in benzene. This solvent did not elute the poison, which was removed by introduction of a little ether, with appreciable concentration.

Discussion

Concentrations of the poison from toxic pasture at times reached the level of 200,000–400,000, and, in one case, went up to 2 million. The latter concentrate must have been largely sporidesmin. The methods followed were not reliable enough to use as a routine. The methods for making concentrates from pasture up to the 70,000 level have subsequently proved reliable, except that, with a few very toxic samples, the poison was not recoverable from the alumina V column.

The application of some of the pasture concentration techniques to wet cultures and to spores produced clean concentrates, usually with some loss of poison, and with a toxicity-to-weight ratio greatly exceeding that possible with pasture. This is an argument in favour of identity of the toxic component of pasture and of fungal cultures. Two further steps using partition columns, the first giving a significant concentration, the second being a purification step, allowed an apparently pure liver-damaging chemical named sporidesmin to be isolated.

The yield of poison, as judged by the various ways of estimating the toxicity level of the starting material and of sporidesmin, was from 25% to 90% in routine processing. None of the side fractions from concentration procedures produced significant liver damage, even at high levels of feeding.

The main losses of sporidesmin were associated with the acetic acid-washed alumina column, but modifications have reduced these losses to small proportions. Subsequent experience with pasture processing has shown a good recovery of toxicity after alumina, following the original directions. But with cleaner concentrates obtained from wet cultures, and from spores in particular, losses of toxicity were serious. As carotenoids were largely absent from the cleaner concentrates, it was decided to add carotene, and as sporidesmin was known to be sensitive to traces of acid, the washing of alumina was intensified. This modification gave good recovery of sporidesmin from concentrates of wet cultures, but not from the cleaner concentrates from spores. Negligible losses using spore concentrates were achieved only by drastic reduction of the amount of alumina used, and by more thorough washing to remove traces of acidity.

When sporidesmin was obtained in a state approaching purity, its stability was much greater than experience with crude extracts had suggested. The yield of sporidesmin has accounted approximately for the liver-damaging ability of some ether extracts of fungus cultures. There is the possibility that the substance accounting for losses in toxicity on concentration is not in fact sporidesmin. Sporidesmin has not yet been isolated from toxic pasture, but there is no chemical evidence to suggest that the liver-damaging factor of pasture is not in fact sporidesmin, arising from the spores on pasture.

The concentrates after stage (7) were colourless and resinous, and, although apparently pure, failed to crystallise from many solvents tried. However, from solvents containing carbon tetrachloride, the purest concentrates gave beautiful clusters of thin plates, showing birefringence with brilliant extinction colours. Analysis, weight evidence, infra-red and other evidence were consistent with an interpretation of these crystals as sporidesmin with carbon tetrachloride of crystallisation.

Two separate preparations, amounting to 17 and 70 mg of these crystals, have been obtained from cultures, and 170 mg have been obtained from spores.

The melting behaviour, optical rotation, ultra-violet and infra-red spectra, X-ray crystallographic evidence, and elementary composition,

have been presented briefly by Synge and White (1959). Further evidence on the characterisation of sporidesmin, not to be presented at this stage, makes a strong case for its chemical individuality and for a high degree of purity of the various preparations of crystals.

The sporidesmin–CCl_4 complex is soluble in 1,800 parts of water at 25°C, less soluble in petroleum ether, and readily soluble in most organic solvents.

The resinous forms and crystalline preparations of sporidesmin, when fed to guinea-pigs by the standard technique, have given mild to moderate liver damage at the 50–100 μg level, and generally severe damage at the 100–200 μg level. The toxicity to HeLa cells is extremely high.

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THE REPRODUCTIVE PERFORMANCE OF EWES MATED ON LUCERNE

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Summary

Trials were conducted to make a comparison between the reproductive performances of ewes de-pastured during mating on lucerne, on the one hand, and on rye-grass-white clover, on the other. In general, the lucerne was grown on thinner soils than was the pasture.

These trials showed that, under the soil and climatic conditions operating, reproduction was depressed by lucerne relative to grass to the extent of:

(1) a retardation in rate of conception of the order of 10%–15% fewer ewes settling during the first 17 days of mating and a 4–5 day retardation of mean lambing date;

(2) a decrease in the number of twins born of the order of 10%, and an increase in barrenness of 1%–2%, giving an overall reduction in lambing percentage of 10%–12%.

Although the factor in the lucerne responsible for these effects was not identified, it is presumed to be the presence of one or more oestrogens; a deficiency of selenium is suggested as another possible causative factor.

Introduction

There have been a number of isolated reports in New Zealand that ewes which grazed on lucerne and red clover during the mating period subsequently gave a disappointing lambing percentage. As far as New Zealand is concerned these reports have come from farm flocks in which the details are meagre and on which no control mob has been kept. Overseas investigations would suggest that oestrogenic activity of the lucerne and clover could possibly account for these observations. Many plants, including pasture species, have been recorded as possessing this activity and it tends to be highest in the legumes.

The extreme case of oestrogenic activity is attained in subterranean clover, which, under certain conditions, has been shown in Australia to cause female sterility, dystokia, and uterine prolapse (Bennetts *et al.* 1946). From such clover a proestrogen, genistein, has been isolated (Curnow *et al.* 1948).

In the United States ladino clover has been under suspicion in this connection. Engle *et al.* (1957) showed that ewes grazed on ladino clover and birdsfoot trefoil conceived 22 and 20 days respectively later than those on bluegrass. First oestrus was displayed later on the legumes than on the bluegrass. Sanger and Bell (1959) confirmed by vaginal smears on these same ewes that oestrogenic activity of the ladino clover and trefoil was involved and that the

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delayed conception was due principally to reduced fertilisation of eggs shed. An oestrogen coumestrol has been isolated from ladino clover by Bickoff *et al.* (1957), who believe that this is the oestrogen present also in lucerne. From red clover two isoflavones–genistein and biochanin A—have been isolated by Pope and Wright (1954).

The oestrogenic activity of lucerne at different stages of growth has been shown by Pieterse and Andrews (1956) to be very variable, while Stob *et al.* (1957) demonstrated a very wide range of potency in different strains of lucerne. However, it would appear that, generally, lucerne is lower in oestrogens that ladino clover and red clover. No reference has been found in the literature to lucerne causing disturbance to the reproductive performance of animals, though the presence of oestrogens suggested that such an occurrence was not impossible.

The purpose of this work was to determine whether or not lucerne could interfere with normal reproductive performance, and, if so, to what degree and in what manner.

Experimental

Outline

The trials extended over a three-year period, 1957-59 inclusive, in the following sequence:

1957. Two groups each of 306 ewes were flushed and mated on lucerne, and on pasture as the control. Lucerne depressed the lambing percentage.

1957a. In an experiment not designed to study the effect, 87 ewes mated on lucerne gave a lower percentage than 260 mated on grass.

1958. The 1957 experiment was repeated and the 1957 results confirmed.

1959. Two groups each of 305 ewes were both mated on lucerne. Selenium was administered to one group, but without effect.

1959. As further circumstantial evidence, a mob of 600 ewes mated on grass gave a significantly higher lambing percentage than the 1959 ewes mated on lucerne.

Of the five trials three (1957, 1958, 1959) were specifically designed to study the problem; three other experiments—1955a, 1957a, and 1959a—present supplementary evidence. With the exception of the 1957a trial, which was carried out on the Research Farm at Canterbury Agricultural College, all the trials were conducted on the Ashley Dene farm. In most, but not all, instances the lucerne was of the New Zealand Marlborough variety, N.Z. certified strain.

Procedure and Results

The Ashley Dene farm of the College occupies a total of 878 acres, of which the majority is thin stony soil. At the time of the

experiments 300-350 acres of the farm were in lucerne only, or lucerne-cocksfoot-sub. clover pastures in which, during mating in the autumn, the lucerne was dominant. The farm carried approximately 1,600 Corriedale ewes, 800 first-cross Border Leicester-Corriedale ewes, and 600 replacement ewe hoggets.

The soil types on the farm consisted of thin stony soils on the Main block of 500 acres, improving to a deep sandy loam on the Cemetery Block of 250 acres, through to a deep heavy silt loam on the Homestead Block of 125 acres. Prefixes M, C, and H to paddock numbers in the text refer to the three Blocks.

An unavoidable detraction from the objectivity of the trial was that the lucerne was on the Main and Cemetery Blocks only. During the dry summer and autumn, grass does not grow in this environment, and so it was not possible to compare lucerne and grass on the same soil type. To get grass in sufficient quantity to match the feed supplied by the lucerne it was often necessary to use paddocks on the Homestead Block. This undoubtedly complicated the series of experiments. One had either to compare green lucerne with dry stalky pasture on the same soil type, which would undoubtedly have introduced the complication of bodyweight change, or to compare green lucerne on the thin soils with green grass on heavier soils. The latter course was chosen.

1957 Trial

For this trial all the two-tooth ewes were employed, consisting of 370 Corriedales and 242 Border-Corriedales. On 13 March 1957 the Corriedale ewes were randomised into two equal groups for flushing and mating on lucerne and on grass. Mean liveweight was 109 lb. Prior to 13 March these sheep had been on grass. Two weeks later, on 27 March, the Border Corriedale ewes, which had been on lucerne for the previous two weeks, were randomised into the two groups and added to the Corriedales. Five Down rams were put out with each group on that date. The control group, during flushing and mating, grazed a succession of perennial rye-grass-white clover paddocks, C7, H5, and H7. The lucerne group, during flushing and the first four weeks of mating, was on lucerne containing some cocksfoot and *Phalaris tuberosa* in paddock C9. Subsequently these sheep were transferred to C8 and C2, in which appreciable quantities of rye-grass were mixed with the lucerne. The mean liveweight

TABLE 1. Liveweight Changes (lb) During Fortnightly Periods from Commencement of Mating

Pre-Mating		Mating		
Group	—2 to 0 Weeks	0–2 Weeks	2–4 Weeks	4–6 Weeks
Lucerne	+ 0.8	+ 8.2	+ 1.8	— 1.0
Grass	+ 0.5	+ 5.3	+ 1.4	+ 1.4

changes during the pre-mating and mating periods are given in Table 1. The changes are the actual gains or losses during each two weeks, from which cumulative gains or losses over longer periods may be calculated.

The two weeks' pre-mating period cannot be considered as flushing in the recognised sense of the term; in fact, only in very exceptional years would it be possible to get liveweight gains of 15-20 lb in 4-6 weeks at this time of year in this environment. Part of the fluctuation in liveweight must be attributable to belly-fill. Nevertheless, the Table shows the changes in the two groups to be approximately the same.

The rams used were raddled, the colour being changed every 17 days. Ewes marked were recorded once a week and at the end of each 17 days. After six weeks of mating the two groups of ewes were joined into one mob, all the rams replaced by three fresh ones, and all the sheep run on grass for a further two weeks, until 22 May. The ewes were wintered and lambed together in one mob. The mating pattern is given in Table 2.

TABLE 2. Mating Records

	Corriedales		Border-Corriedales	
	Lucerne %	Grass %	Lucerne %	Grass %
Ewes served, 1st 17 days	80	88	80	90
Ewes holding to service, 1st 17 days	61	72	66	73
Ewes returning, once	16	16	16	17
Ewes returning, twice	3	2	2	0

Table 2 shows that the ewes in the lucerne group were slower taking the ram, but there were no differences in ewes returning to service. The mean date of holding to service was three days later

TABLE 3. Lambing Records, 1957

	Corriedales		Border-Corriedales	
	Lucerne	Grass	Lucerne	Grass
No. ewes at lambing	183	184	121	120
No. ewes lambing, first 17 days	123	138*	66	79*
No. ewes barren	11	11 NS	7	4 NS
No. ewes single bearing	156	147	96	80
No. ewes twin bearing	16	26*	18	36**
Mean lambing date	7 Sept.	3 Sept.	8 Sept.	4 Sept.
Percentage lambs born/ewes lambing	109	115	116	131

NS Difference not significant.
* Difference significant at P = 0.10.
** Difference significant at P = 0.01.

in the lucerne group. The Corriedales and Border-Corriedales behaved similarly in these respects.

The lambing data are presented in Table 3.

These lambing records confirm the mating data in as far as they show that the lucerne group was, on average, about four days later in lambing. On the other hand, these records show that the Corriedales lambed faster, and the Border-Corriedales slower, than the mating data would suggest they should.

Overall, the lambing percentage of lambs born to ewes mated was lower in the lucerne group by 6% in the Corriedales and 18% in the Border-Corriedales.

1958 Trial

The mob of first-cross Border Leicester-Corriedales was used in the 1958 trial: the 604 sheep were of mixed ages. On 18 February 1958 each age category was separately randomised into lucerne and grass groups. The 302 ewes of the lucerne group were put on a lucerne-cocksfoot pasture in paddock M14, the lucerne being approximately one foot in height and in flower and containing some cocksfoot. The ewes in the other group were put on a perennial rye-grass, Yorkshire fog, white clover pasture, in H3.

Two weeks later, on 5 March, three raddled Dorset Down rams were put out with each mob, followed 10 days later by a further two rams each. The grass group remained on paddock H3 for the pre-mating period and first six weeks of mating. The lucerne group spent the pre-mating period and the first two weeks of mating on M14, after which they were rotationally grazed round a number of three-acre lucerne plots and finally returned to M14. On 14 April the two groups were joined and left on pure lucerne in C5 with five rams until 30 April.

The mean liveweight changes in fortnightly periods are given in Table 4. Mean commencing weight was 135 lb.

TABLE 4. Liveweight Changes (lb) in Fortnightly Periods from Mating

Pre-Mating	Mating		
Group	-2 to 0 Weeks	0 - 2 Weeks	2 - 4 Weeks	4 - 6 Weeks
Lucerne	+ 0.2	+ 6.8	— 3.3	+ 5.2
Grass	— 4.4	+ 6.7	— 5.2	+ 3.6

The liveweight changes favour the lucerne group, but neither group, except during the first fortnight of mating, made sufficiently rapid gains to constitute what is normally meant by flushing.

The mating records are summarised in Table 5.

TABLE 5. Mating Records

	Lucerne %	Grass %
Ewes served, first 17 days	44	61
Ewes holding to first service	36	54
Ewes, served, second 17 days	62	47
Ewes holding to second service	55	42
Ewes served, third 17 days	8	3
Ewes returning once	15	10
Ewes returning twice	1	1

The lambing followed the mating pattern with unusual exactitude. The main points are summarised in Table 6.

TABLE 6. Lambing Records, 1958

	Lucerne	Grass
No. Ewes at lambing	300	301
No. ewes lambing first 17 days	108	162**
No. ewes barren	17	11 NS
No. ewes single bearing	178	163
No. ewes twin bearing	105	127*
Mean lambing date	18 Sept.	13 Sept.
Percentage lambs born/ewes lambing	137	144

NS Not significant.
* Difference significant at P = 0.10.
** Difference significant at P = 0.01.

These records confirm that the mating on lucerne has retarded oestrus; delayed lambing by a mean of five days; increased the percentage of barren ewes by 2%; and decreased the percentage of twins by 7%.

1959 Trial

At the beginning of 1959 the possibility presented itself that the depression in reproductive performance recorded on lucerne was due to selenium deficiency in the lucerne. The reasoning behind this supposition will be mentioned in the Discussion.

It was therefore decided that, in this 1959 trial, two groups of ewes, one given selenium and the other acting as control, would both be mated on lucerne. Accordingly, on 2 March 1959, a mob of 610 mixed-age Corriedale ewes, including 50 two-tooths, was taken; each age group was separately randomised into two equal groups. Each ewe in the selenium group was given an injection of 5 mg selenium in the form of sodium selenate. All the ewes were grazed in paddock C9 on a lucerne-dominant pasture (including some cocksfoot and phalaris) for three weeks until the rams were turned out on 23 March, on which date a further injection of selenium was

given. For the whole mating period the ewes were on a succession of pure lucerne or lucerne-dominant pastures in C2, C8, and C9. A third and final selenium injection was given on 23 April. The rams were removed on 12 May.

The most severe drought for over 40 years had been experienced in the summer of 1958-59, so that the ewes were in poor condition. Mean bodyweight in the selenium and control groups was 89.8 and 89.5 lb on 2 March, and had increased to 97.8 and 96.5 lb at the time the rams were joined with the ewes three weeks later. The sheep continued to improve as mating proceeded, but further bodyweights were not taken.

The mating and lambing records are summarised in Table 7.

TABLE 7. MATING AND LAMBING RECORDS OF EWES MATED ON LUCERNE, 1959

(305 ewes per group)

	Selenium %	Control %
Ewes served, first 17 days	80	80
Ewes settled, first 17 days	64	63
Ewes served, second 17 days	20	21
Mean lambing date	30 Aug.	29 Aug.
Ewes lambed, first 17 days	68	72
Ewes barren	4	4
Lambs born/ewes lambing	126	128

It is clear from these figures that the administration of selenium had no effect on reproduction. None of the differences between treatments is statistically significant.

Supplementary Evidence

In the course of other experimental work over the years there have been occasions when one randomised group of a mob has been mated on lucerne and the others on grass. Although these experiments were not designed to study the lucerne effect, they do add further evidence to the problem. The following experiments represent all, and not just a selection, of trials on which there is documented evidence.

1959a. On 9 March 1955 a mob of 450 mixed-age Border-Corriedale ewes was randomised, each age separately, into three mobs, each consisting of 150 ewes; three rams were put with each group until 27 April. One of the groups was mated on a grass paddock (M3), while two each had one half of a lucerne-dominant pasture (C9). No mating records were kept, but all lambs were recorded at birth.

The number of lambs born per ewe lambing was 168% in the grass-mated group, and 156% and 161% in the lucerne groups. When the numbers of twin- and single-bearing ewes were tested by Chi square the 168-156% difference reached statistical significance at the 5% level. The number of barren ewes and the mean date of lambing were the same in all groups.

1957a. A mob of 350 aged Romney ewes was randomised into four groups and mated as follows:

Group 1—89 ewes, 3 Romney rams, on pure lucerne;
Group 2—87 ewes, 2 Romney rams, on short grass;
Group 3—87 ewes, 2 Border Leicester rams, on short grass:
Group 4—87 ewes, 1 Border Leicester ram, on good grass.

None of the sheep had been on lucerne during the previous few months. This experiment was conducted on the Research Unit at Canterbury Agricultural College on a type of soil that was a silt loam; Group 1 was pastured on lucerne and the remainder on various permanent grass pastures.

Rams were put out with the ewes on 14 March. On 26 April, by which time 90% of the ewes were in lamb, the four groups were boxed together with the Border Leicester rams only for one week on lucerne, followed by one week on grass. The rams were then removed and the ewes wintered on grass and hay. Mean bodyweight at the commencement of mating was 121 lb and gains of the four groups during the mating period were 17 (lucerne), 5, 3, and 19 lb, respectively.

The lambing results are summarised in Table 8.

TABLE 8. Lambing Records of Lucerne and Grass-Mated Ewes

	(1) Lucerne %	(2) Grass %	(3) Grass %	(4) Grass %
Ewes lambing, first 17 days	47	62	51	58
Lambs born/ewes lambing	132	136	140	153
Barren ewes	9	5	7	5

When it is remembered that the weight gain on the lucerne was much higher than in two of the three grass groups it is clear that the lucerne-mated group has not performed as well as the other three groups. The fact that different numbers and breeds of rams were used should not have influenced the percentage of lambs born to ewes lambing. The difference in twin-bearing ewes reaches $P = 0.10$ only if the grass groups combined are compared with the lucerne group.

1959a. The 1959 trial reported earlier was conducted in a mob of mixed-age Corriedale ewes mated on lucerne to Border Leicester rams. At the same time, another mob of 662 Corriedale ewes was

mated on grass to Corriedale rams. These two mobs of ewes had had identical treatment during the previous three months, having been run together in one large mob. Rams were joined with the ewes on the same day, 23 March.

The two mobs were, however, not randomised. The grass-mated group contained the better-woolled and more open-faced ewes, which might be expected to have improved performance; but, on the other hand, it included 30% of two-tooths, whereas the lucerne-mated group contained only 8%. The difference that one might expect to find between the lambing performance of the two mobs if they had been mated together is a matter of opinion.

In the event, the grass-mated group gave a lambs born/ewes lambing percentage of 140% as against 127% for the lucerne-mated group, and the percentages of barren ewes were 2.0% and 4.0% respectively.

Oestrogen Assays

It was not possible to do concurrent assays of the lucerne for the presence of oestrogens. However, during 1959 and 1960, Dr D. S. Flux and Mr R. Mumford of Massey Agricultural College, Palmerston North, kindly undertook some assays on the lucerne and the author wishes to acknowledge his appreciation of this work.

The samples of lucerne were taken from the stands which had been used in the flushing trials, namely, M14, C5, and C9, and were freeze-dried.

A sample of lush actively growing lucerne taken in the spring (September 1959) showed no evidence of oestrogenic activity. During the following summer and autumn further samples were taken when the lucerne was in a condition similar to that used in the trials, namely, not actively growing, fairly mature, with some heads in flower, and all slightly wilted during the day. The results of these tests are shown in Table 9.

Samples of Marlborough, Provence, Rhizoma, and Glutinosa strains of lucerne in an actively growing state, taken in March 1960

TABLE 9. Tests for Presence of Oestrogens in Lucerne

Sample	Date Of Sampling	No. Mice Per Feet	Mean Uterine Wt. (mg)
Control	—	6	4.58
Ashley Dene	Dec. 1959	7	5.79*
Ashley Dene (leaf)	Feb. 1960	6	5.08
Ashley Dene (stem)	Feb. 1960	6	5.92*
College Paddock W3	Feb. 1960	7	5.42*
Control (for March 1960 only)		6	5.28
Ashley Dene	March 1960	6	5.83

* Differences from control significant at 5% level.

from College Paddock N5, showed no significant increases over controls.

It appears from these results that, while oestrogen can be detected during the summer-autumn period, in the type of lucerne used in the trials the amount of oestrogen detected is very slight. The possibility exists that the lucerne might contain oestrogens which do not survive the drying processes. But the fact remains that these tests merely show that oestrogenic activity can be detected without proving or disproving that oestrogens could be responsible for the effects observed in the sheep grazing the lucerne.

Discussion

In brief, the results of these investigations show that ewes mated on lucerne have been later in taking the ram, and, consequently, later in lambing, and have produced fewer twins and, possibly, more barren ewes. These effects are not related to the failure of lucerne to sustain body condition, since the weight put on during mating by the lucerne-mated groups was the same as, or more than, that put on by the grass groups. The effect must therefore lie in some specific substance or substances, or in a deficiency in the lucerne. One must reiterate that the lucerne and the grass were not grown on the same soil type, and this difference in soil could influence the comparison. The effect on reproduction might be looked at in slightly more detail.

In the 1957 trial the ewes in the lucerne group were flushed as well as mated on lucerne, and the rams were introduced at a relatively late date (27 March), by which date one would expect all the ewes to be cycling. The ewes probably were, in fact, cycling, since in both groups 80-90% of ewes were served in the first 17 days, 60-70% holding to service and subsequently 60-70% lambing in the 17 days. The difference between the lucerne and grass-mated groups was mainly that, in the lucerne group, approximately 10% fewer ewes took the ram, settled to service and lambed to the first 17 days, which in turn was reflected in a later lambing. The percentage of ewes returning to service did not appear to be affected.

In the following year the rams were introduced on 5 March before many of the ewes had had their first oestrus, and, consequently, the percentage served, settled, and lambing in the first 17 days, was considerably less. Under these circumstances the lucerne seemed to produce an even greater retarding effect on conception, 15-20% fewer ewes being served, holding to service, and lambing to this period. The 1957a supplemental trial, but not the 1955a trial, confirmed these findings. The magnitude of the effect on rate of conception and lambing would therefore seem to be 10-15% fewer ewes settling to service and subsequently lambing in the first 17 days, and a consequent retardation of mean lambing date by 4-5 days.

Barrenness

Strangely enough, barrenness seems to be the least affected of all aspects of reproduction. The increase in percentage of barren ewes

in the lucerne group was 1.5%, 2.0% 0.0%, and 3.0%, in the 1957, 1958, 1955a, and 1957a trials, respectively. One could postulate that this increase was due to something rendering the ewes infertile; and, while this may indeed be the case, the smallness of the differences suggests that they are merely the end-results of retarded conception, and that, had the rams been left with the ewes long enough, the differences would have disappeared. In this respect the results agree with those of Engle *et al.* (1957).

Twinning Rate

In all the experiments recorded the depression in percentage of twins in the lucerne group has been one of the most consistent features, being 10%, 7%, 10%, and 10% for the 1957, 1958, 1955a, and 1957a trials, respectively. While these differences individually just fail to reach statistical significance at the $P = 0.05$ level, the combined results leave little doubt that the effect is a real one.

One postulate is that the depression in reproductive performance is due to oestrogenic activity in the lucerne. An alternative theory offered itself in 1959 when the use of selenium to counteract white muscle disease in lambs was introduced. It had been observed that selenium had reduced the incidence of barrenness on one farm on which white muscle disease had occurred and which had lucerne; that the disease had occurred on Ashley Dene; and that the disease tended to occur more frequently on farms which had lucerne. The possibility existed, therefore, that lucerne, but not grass, might be deficient in selenium. Consequently, the 1959 trial was carried out, with control and selenium-treated ewes mated on lucerne; however, this trial produced negative results. These negative results, however, cannot be regarded as conclusive because, in this particular year (1959), the incidence of white muscle disease in the lambs at Ashley Dene was low.

At the present time, therefore, the cause of the lucerne effect cannot be defined. Oestrogen assays on the lucerne during the years of the trials were unfortunately not made, owing to lack of facilities. Tests made subsequently have proved the presence of oestrogenic activity in the lucerne, but the activity as measured was of a low order. The observations on the sheep are consistent with either a decreased ovulation rate or decreased fertilisation, and do not distinguish between these two possibilities. Twinning rate appears to be much more sensitive to the lucerne than does barrenness. Sanger and Bell (*loc. cit.*) showed that a lower percentage (59% as opposed to 75%) of eggs was fertilised 72 hours after mating from ladino-pastured, than from grass-pastured, ewes. It would appear that, when oestrogenic activity is really high, as in the Australian sub. clover experiments, barrenness is the ultimate and most spectacular effect of oestrogen.

It has been assumed that the major influence of the lucerne has been on the ewe. This view is supported by the fact that ovulation rate as measured by number of lambs born per ewe lambing, rather than barrenness, contributed so largely to the differences observed.

But one cannot be certain that this is the only influence. The slower rate of conception, and retarded lambing, could be attributed to either the ram or the ewe or both; but the fact that the ewe is already involved leads one to suspect it, rather than the ram, in this connection also.

One is led to the conclusion that what has been observed in the present series of trials is a marginal effect. It would not have been observed by farmers in the ordinary course of events. The lambing percentages and the percentage of barren ewes in the lucerne-pastured groups were not abnormal by ordinary New Zealand farming standards, and it has required detailed experimentation to demonstrate the depression in reproductive performance of the order of 10%. In addition to the reports of some disappointing lambing percentages which led to this investigation, it must also be said that some farmers who use lucerne extensively have very good lambing performances: one farmer, in particular, has an exceptionally good record. While some of these differences may be due to management, it would appear that there is variation in the lucerne itself. At present it is not known at what stage the lucerne does or does not influence reproduction. Since soil type, climatic conditions, stage of growth, and strain of lucerne must all influence the power of lucerne to affect reproduction, the number of variables is so great that much work would be needed to define the conditions under which lucerne should not be grazed.

It would be unfortunate if the results of these trials were misinterpreted. Lucerne has played such an important part in increasing productivity on the drier areas of the South Island that the results presented in this paper should not be allowed to impede the further extension of lucerne growing. In the typical dry summer and autumn period lucerne is often the only species which provides good grazing for sheep prior to mating. The trials have shown that, if an alternative feed, such as grass, can be provided, it would be better to use that rather than lucerne. But this alternative does not always exist; and one does not know what the results would have been had the comparison been between lucerne and whatever else was available, which, in most cases, would have been dry stalky pasture insufficient to maintain bodyweight. The results of the trials merely show that the grazing of lucerne immediately before and during mating can result in a reproductive rate which is less than would be expected, and that plant breeders and farmers should be aware of this possibility.

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CAROTENOIDS AND VITAMIN A IN THE BLOOD OF THE BOVINE

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Summary

This paper summarises results of carotenoid and vitamin A alcohol assays on a large number of individual blood samples taken from groups of pasture-fed dairy cows throughout the 1954–55 to 1958–59 dairying seasons. Marked seasonal variations are shown to occur each year and attention is particularly drawn to the very large variability in the blood levels of carotenoids and vitamin A alcohol in individual animals of the same breed.

The effect, under pasture-feeding conditions, of factors such as breed, age, sex, and lactation on blood carotenoid and vitamin A alcohol levels is also reported. It is shown that, under New Zealand conditions of high carotene intake throughout the year, the relationship between blood and milk-fat carotenoid levels is very different from that reported by overseas workers for lower carotene intakes.

Introduction

Various aspects of the carotenoid metabolism of dairy cows have been under active investigation in this Department for a number of years. Much of the work has centred round the levels of carotenoids and vitamin A in the blood of cows and a very large number of assays for these components have been carried out on blood samples taken from individual animals at intervals throughout dairying seasons. Much of this work has already been reported elsewhere (McGillivray 1957a, b, c, d, 1959; Thompson and McGillivray 1957; McDowall, McGillivray, and Reid 1957; McGillivray, McDowall, and Reid 1959). However, during the course of the investigations, it became apparent that the figures obtained for control cows grazed normally on typical dairy pastures, and for other cows subjected to treatments which clearly had no effect on their carotenoid metabolism, would provide additional information about the levels of carotenoids and vitamin A in the blood of typical dairy cows in New Zealand and about some of the factors which influence these levels.

Blood carotenoid and vitamin A levels in the bovine have been investigated by a number of workers. Their findings, some of which have been reviewed recently by Deuel (1957), Moore (1957), and McGillivray (1961), indicate that the carotenoid content of the feed is the main factor influencing the levels of these constituents in the blood. Diet has a more marked effect on carotenoids than on vitamin A alcohol in the blood, the latter being normally maintained at a fairly uniform level through release of stored vitamin from the liver. The only immediate effect of diet is on the ester fraction of the vitamin A in the blood, but this fraction makes up only a small proportion of the total

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amount of vitamin A in the blood of the pasture-fed bovine (McGillivray 1957a). The effect of feed on blood carotene is particularly apparent where cows are transferred from low-carotenoid winter rations to spring pastures; Lord (1945), for example, quotes average carotene figures from as low as 130 μg/100 ml plasma during the stall-feeding period to as high as 1,680 μg/100 ml for cows on pasture. Even while cows are pasture-fed, their blood carotenoid levels are still susceptible to changes in carotenoid intake resulting from drying-up of the pasture during mid-summer (e.g., Sutton and Soldner 1945). Under New Zealand dairying conditions, however, a different situation exists, and there is normally available to dairy cows, throughout the season, an adequate supply of lush green feed of high carotene content (McGillivray 1956). Nevertheless, marked variations in the carotenoid and vitamin A content of the blood of a group of typical pasture-fed cows have been reported previously by McGillivray (1957a).

Besides diet, other factors have been shown to have a lesser, but still significant, effect on bovine carotenoid and vitamin A blood levels. The effect of breed, particularly on the carotenoid content of the blood, is well recognised. Of the more common breeds, Guernseys appear to have the highest levels of carotene in their blood; on the same feed Jerseys reach about 60% of the Guernsey figure, and Holsteins and Ayrshires about 50% and 45% respectively (e.g., Sutton *et al.* 1945; Wise *et al.* 1947). Age has a marked effect on carotenoid levels in the blood of the bovine, the young animal being born with only traces of carotenoids in its blood (Moore and Berry 1944). The rate at which normal adult levels are attained might, however, be expected to depend on the daily carotene intake of the animals. Various workers have found marked differences between the levels of carotenoids in the blood of cows and bulls (Gillam and El Ridi 1937, 1945; Sutton and Soldner 1945), but it is uncertain whether these differences are related to sex or merely to dietary factors associated with the different management of cows and bulls. The onset of lactation causes a sudden drop in blood carotenoids and vitamin A (e.g., Walker *et al.* 1949). This decrease in blood levels has been confirmed even under pasture-feeding conditions and is due to the abnormally high uptake of carotenoids and vitamin A by the mammary gland at the start of lactation (Thompson and McGillivray 1957; McGillivray 1959). Once lactation is established there is no evidence of its effect on blood levels.

Experimental

All animals were pasture-fed throughout the year and were maintained according to the best husbandry practices normally obtaining in New Zealand. They were all from the herd of The Dairy Research Institute (N.Z.) and the figures quoted relate in general to one animal of a pair of monozygotic twins. Since there is no discrimination for breed or other characteristics in the collection of identical twins for the Institute herd, it seems likely that the animals included in this survey represent a fair cross-section of dairy cows in the Manawatu and surrounding districts.

The five-year survey covers assays made on blood samples taken from a total of 62 individual cows. Assays on 36 of these were restricted

to one season only; 21 cows were followed through two seasons, and five through three seasons. Forty-three of the cows were pedigree or "high-grade" Jerseys; 17 were Jersey crosses with Friesians, Ayrshires, Shorthorns, and Black or Red Polls; 2 were pedigree Ayrshires. Assays did not extend throughout the season in all cases; as is apparent from the data presented, attention was focused on the September to January period when blood levels had been found to change most rapidly.

In the case of carotene, just under 1,000 assays, spread fairly uniformly over the five years, have been included in the five-season survey. Approximately the same number of assays are presented for xanthophyll and vitamin A alcohol, but there were a few occasions when, for various reasons, all three constituents were not estimated in particular blood samples. No data are included for vitamin A ester. Although present in the blood of pasture-fed cows, preliminary work (McGillivray 1957a) indicated that the levels were too low for accurate assay unless inconveniently large amounts of blood were extracted.

In addition to the above data relating to normal, pasture-fed, lactating cows, opportunity was taken, whenever suitable experimental material was available, to investigate the influence of various factors on blood carotenoid and vitamin A levels. During one season the effect of lactation was investigated using five pairs of twins where only one animal of each pair was lactating, the other having failed to get into calf the previous year. During another season the levels of carotenoids and vitamin A in the blood of six heifers were followed to determine whether these younger animals showed seasonal trends similar to adult animals. To investigate the effect of sex, when both cows and bulls were under similar dietary conditions, blood samples were taken at intervals from two pasture-fed pedigree Ayrshire bulls and two pedigree Ayrshire cows.

In many cases milk samples were taken at the same time as the blood samples. Data from these were used to investigate the relationship between the levels of carotenoids in blood and milk fat.

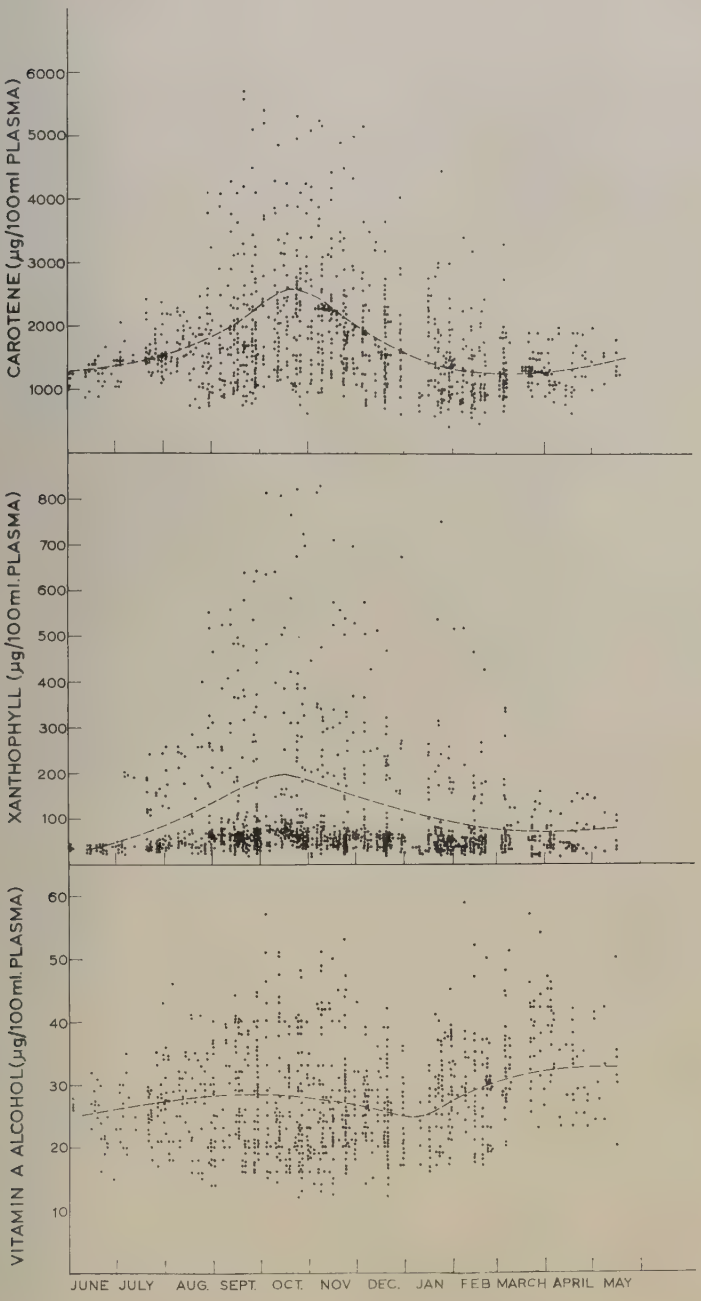
With most animals, blood samples were taken at weekly intervals, but, in a few cases, samples were taken at intervals which ranged from 2–3 days up to one month. Carotenoids and vitamin A alcohol were determined in blood and milk samples using the method of Thompson, Ganguly, and Kon (1949).

Results and Discussion

Seasonal Trends

Seasonal trends in the levels of carotenoids and vitamin A alcohol are shown in Fig. 1. The curve is, in each case, the average of all the assays carried out over the five dairying seasons. With the carotenoids the same trends were apparent in the curves for each individual season: a steady rise to maxima in October, thereafter a decline to minima in late summer, followed by slight increases in late autumn. The data

Fig. 1.—Seasonal changes in the carotenoid and vitamin A alcohol levels in the blood of 62 individual cows throughout the five dairying seasons 1954–55 to 1958–59.


CAROTENE (µg/100ml PLASMA)
6000
5000
4000
3000
2000
1000
XANTHOPHYLL (µg/100ml. PLASMA)
800
700
600
500
400
300
200
100
VITAMIN A ALCOHOL (µg/100ml. PLASMA)
60
50
40
30
20
10
JUNE JULY AUG. SEPT. OCT. NOV DEC. JAN FEB MARCH APRIL MAY

obtained in this survey suggest a very uniform pattern for both carotene and xanthophyll each season, with the curves for any individual season displaced by up to ± 2 weeks from the average curves shown in Fig. 1, and with some slight differences in the rate of change in the carotenoids, particularly the rate of decline during November. This decline would appear to be related to climatic conditions: an early dry spell, with associated hardening of the pasture, caused an earlier and steeper-than-normal decrease in both carotene and xanthophyll; whereas a wetter spring, with extended growing period, was associated with a later and less rapid decline.

The average ratio of carotene to xanthophyll (expressed as μg carotene divided by μg xanthophyll) is shown in Fig. 2. It is apparent that, on the average, the concentration of carotene is 15–20 times that of xanthophyll, with an indication of a seasonal trend, the highest ratios occurring in early spring and late autumn.

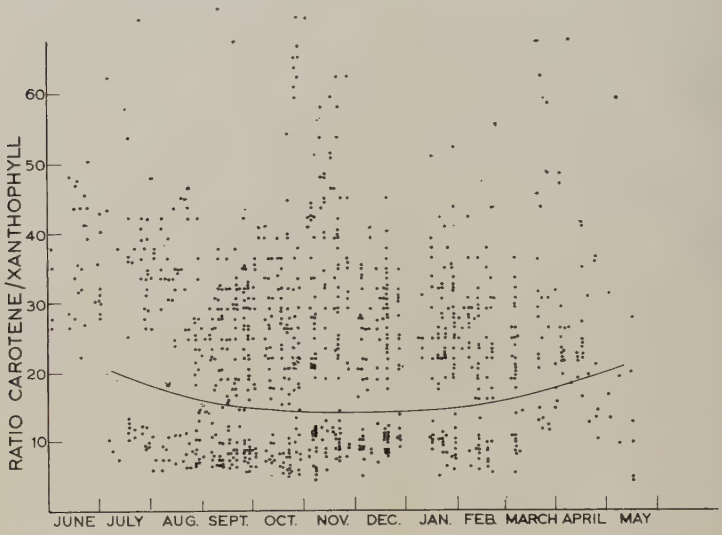


Fig. 2.—Ratios of carotene/xanthophyll in the blood of individual cows.

The seasonal trend in carotenoid levels shown in Fig. 1 is similar to that reported previously for a group of pasture-fed cows (McGillivray 1957a), but the present five-year average is about 35% higher throughout the year. This difference is not surprising, since the previously published figures related to six cows only; but, even with the large numbers of cows involved in the present survey, the average carotenoid levels differed markedly from season to season. This is demonstrated in Fig. 3, in which average maximum and minimum carotenoid levels for the five seasons are shown. The general upward trend indicated by all the curves apparently resulted from the changing populations available from season to season, since the average age of the groups remained unchanged from season to season, and, as is discussed later, individual animals

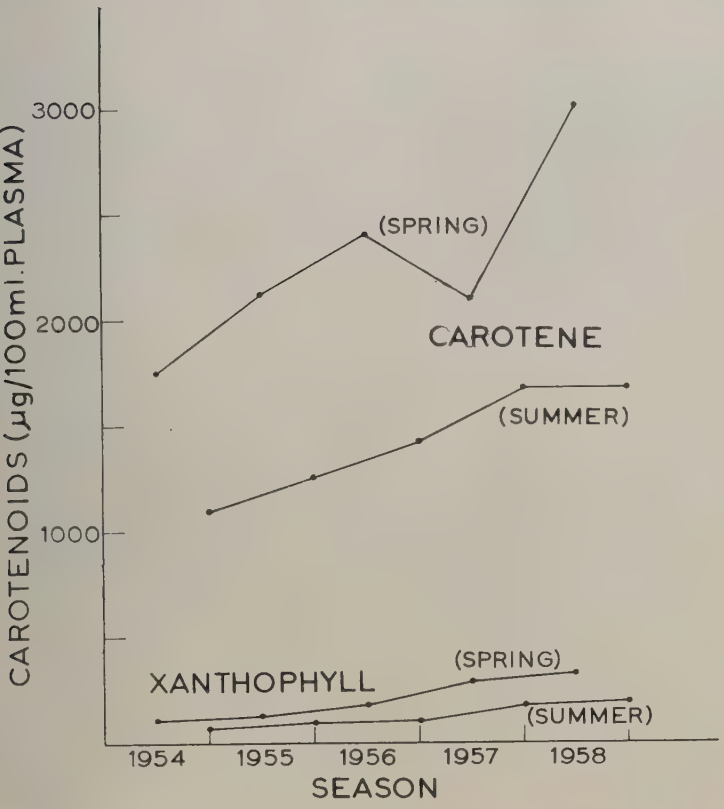


Fig. 3.—Average levels of carotenoids in the blood of groups of cows during spring (maximum) and summer (minimum) months from 1954 to 1959.

followed through more than one season were remarkably constant in the levels of carotenoids in their blood.

The picture with vitamin A alcohol was not so consistent from season to season as for the carotenoids. In each season the average amount of vitamin A alcohol in the blood increased slightly during the spring, reaching a maximum during September to November; thereafter it declined rapidly to a minimum in December or early January, then increased again from late January and early February onwards. The trends are apparent in Fig. 1; but, because of the variations from season to season, particularly in the period over which the maximum and minimum concentrations occurred, the averaging of the results has had the effect of smoothing out some of the more pronounced variability apparent in the data for individual seasons. The average maximum and minimum values recorded each year were of the same order as those already reported for a group of six cows during the 1954–55 season (McGillivray 1957a).

Breed

As already noted, 17 of the cows were Jersey crosses. However, because of the number of other breeds involved in the cross-breeding and the wide variability in blood carotenoid levels between animals of the same breed, the present data provide little information about the effect of breed on blood carotenoid and vitamin A alcohol levels. If any conclusions can be drawn, it would seem that their Jersey ancestry markedly influenced the carotene metabolism of the cross-breeds and the blood carotene levels in these animals were fairly evenly distributed through the normal Jersey range. Xanthophyll levels were, however, all very low, giving much higher carotene/xanthophyll ratios than were normal for Jerseys. Carotene and xanthophyll levels in the blood of the pedigree Ayrshire cows were relatively low, being, throughout the season, about half of the Jersey average. Vitamin A alcohol levels bore no relationship to breed.

Individuality

The great variation in the levels of carotenoids and vitamin A alcohol in the blood of individual cows is clearly apparent from the wide scatter of points in Fig. 1. Each point relates to one determination on one animal. In order to present all results, a few points have been displaced by up to $\pm$ 2 days from the actual sampling date. The effect of this on the general pattern, however, is insignificant. The data presented relate to cows of all breeds, but, as already mentioned, cross-breeds, apart from their xanthophyll level, fell within the normal Jersey range. However, restricting consideration to Jerseys alone, the difference in blood carotene levels frequently exceeded five-fold. Thus, during the spring months, several cows exceeded 5,000 μg/100 ml plasma, whereas, at the same time, a number of cows were below 1,000 μg/100 ml. The greatest difference recorded on any one sampling day was just under five-fold, one Jersey having 5.200 μg carotene/100 ml plasma and another only 1,050 μg/100 ml. The highest level recorded was 5,700 μg/100 ml plasma. On two occasions in midsummer, values below 500 μg/100 ml plasma were recorded for Jerseys.

In the case of xanthophyll, the distribution is somewhat different from that of carotene. Only a relatively small proportion of the carotenoids in the blood of most Jerseys was xanthophyll and approximately two-thirds of the results were below 100 μg/100 ml plasma. Higher carotene contents tended, however, to be associated with higher xanthophyll levels, and, in some cases, the carotene to xanthophyll ratio was as low as 5.0. The highest xanthophyll level recorded was 830 μg/100 ml (corresponding carotene level-5.200 μg/100 ml; carotene/xanthophyll ratio–6.3). On the same sampling day the level of xanthophyll in the blood of another Jersey was only 50 μg/100 ml plasma (corresponding carotene level–2,100 μg/100 ml; carotene/xanthophyll ratio–42).

In the majority of cases blood vitamin A alcohol levels bore no relationship to carotenoid levels, except that very high or very low vitamin A alcohol levels (above about 45 μg/100 ml plasma and below about 15 μg/100 ml) were frequently associated with similar extremes in carotenoids. The highest value recorded was 59 μg/100 ml plasma, and, on one sampling day, levels ranged from 57 down to 15 μg/100 ml.

The individual variability between animals of the same breed is the most striking feature of this survey. It indicates marked differences in the carotenoid and vitamin A metabolism of individual animals, although they may be on identical feed and under similar conditions of management. In the case of the carotenoids, the explanation for these individual differences may lie in their initial absorption from the intestine, in the efficiency of conversion of absorbed carotene to vitamin A, or in the rate of destruction or removal from the blood of unchanged carotenoids. There are, of course, clearly recognised species and breed differences in carotenoid metabolism (Goodwin 1952), and, in all breeds of cattle, there is a marked discrimination in carotenoid absorption in favour of carotene, so that it is perhaps not so surprising that individual differences should be found within one breed. It is clear, however, that these between-animal differences in carotenoid metabolism greatly exceed the recognised breed differences and also outweigh many of the other factors known to influence blood carotenoid levels.

The blood level of vitamin A alcohol, as distinct from the ester form, is not directly related to vitamin or provitamin absorption, so that no correlation would be expected between the blood levels of vitamin A alcohol and carotenoid. The former would represent a balance between the vitamin A released from the liver and that taken up by the tissues. The wide range of values for normal animals on carotene-rich feed suggests that, in vitamin A studies, greater significance attaches to changes

TABLE 1. Blood Levels of Carotenoids and Vitamin A (Spring Averages) for a Selection of Individual Cows (expressed in μg/100 ml plasma)

Cow	Approximate age when first sampled (Years)	Season 1				Season 2				Season 3			
		Carotene	Xanthophyll	C/X Ratio	Vitamin A Alcohol	Carotene	Xanthophyll	C/X Ratio	Vitamin A Alcohol	Carotene	Xanthophyll	C/X Ratio	Vitamin A Alcohol
Individual Animals													
A	4	1320	50	26	27	1350	45	30	25				
B	3	3630	370	9.8	28	3700	400	9.3	26				
C	2	1350	95	14	30	1500	88	17	27				
D	2	1500	90	17	25	1690	110	15	22				
E	2	2500	400	6.3	30	3200	550	5.8	32	3100	500	6.2	33
F	1	2400	400	6.0	45	4950	890	5.6	48	5600	920	6.1	50
Twins													
G1	3	2550	230	11	35	2650	270	9.8	32	2400	250	9.6	36
G2		2400	230	10	38	2650	290	9.1	38				
H1	2	2850	95	30	27	3200	120	27	23				
H2		2800	90	31	27	3150	110	29	22				
I1	1	900	50	18	19	1400	100	14	21				
I2		750	50	15	20	1100	95	12	21				
J1	1	970	47	21	18	1300	75	17	19	1720	90	19	22
J2		750	35	21	20	1200	55	22	20	1500	75	20	20

in blood levels than to the absolute amount present at any one time, since this apparently gives no indication of the vitamin A status of the animal.

Whatever factors do influence blood carotenoid and vitamin A levels within individual animals, it is clear that they are largely genetic in origin and that they do not vary greatly throughout the lifetime of the adult animal. Although the results available here do not lend themselves to statistical analysis for uniformity, it is apparent that identical twin mates, when under similar conditions, are very uniform in their blood levels of carotenoids and vitamin A alcohol. In most cases, where treatments were the same, any differences between twin mates were within the experimental errors of the determinations. Larger differences, where they occurred, were maintained throughout the season

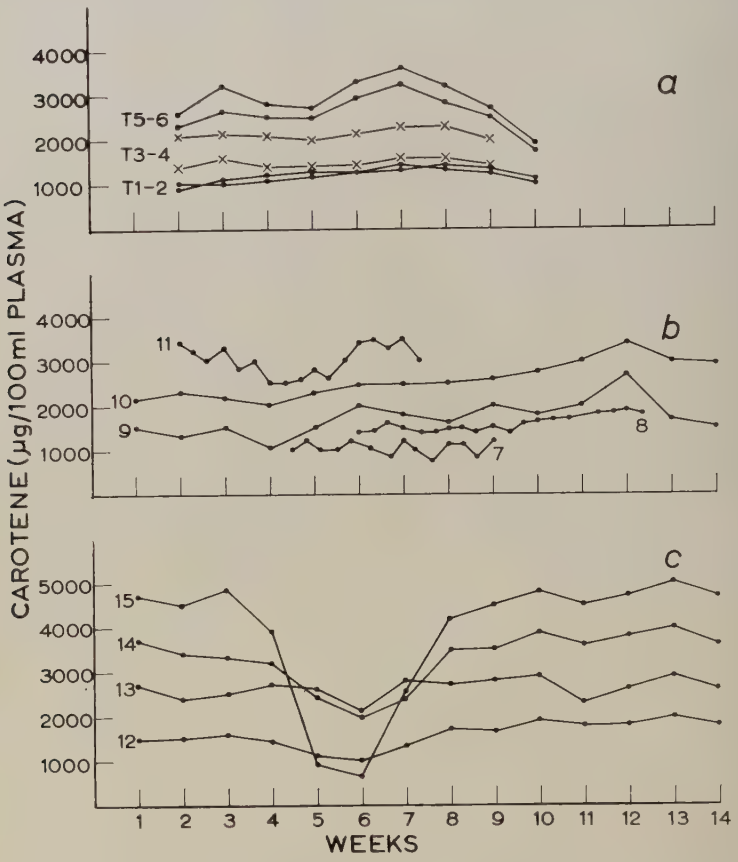


Fig. 4.—Typical sample-to-sample variations in the levels of carotene in the blood of individual cows and monozygotic twins: (a) three pairs of twins; (b) five individual cows; (c) four individual cows. (The three graphs relate to separate sampling periods.)

and from one season to the next. This is illustrated in Table 1 and Fig. 4, which include typical results for pairs of twins. Pair T1-2 in Fig. 4a were typical of most twins; a few pairs were similar to pair T5-6, and one pair (T3-4) consistently showed greater twin-mate differences. Even in this case, however, these differences were maintained throughout the two seasons when the animals were sampled.

As is also apparent from the typical results presented in Table 1, individual adult animals which were followed for more than one year were remarkably consistent in their blood carotenoid and vitamin A alcohol levels from one season to the next. Generally speaking, only the younger animals showed changes of more than about ± 10% in these blood constituents from one season to the next.

In general, the blood levels of carotenoids and vitamin A alcohol for individual cows showed variations similar to the seasonal trends reported above for the whole group of animals. Apart from these clearly defined seasonal trends, many animals were very consistent from one sampling date to the next throughout the season, individual samples frequently showing variations of less than about ± 5% from the steadily increasing or decreasing mean. However, there were occasions where marked fluctuations of an erratic nature occurred. These fluctuations could not be related to any obvious changes in the type of pasture being consumed, nor did they appear to be associated with climatic conditions. Typical fluctuations of the types normally encountered are shown in Fig. 4. Erratic fluctuations are particularly marked in cows 7 and 11 (Fig. 4b), sampled at intervals of 2–3 days. and in cow 9, sampled weekly. They are less marked in cows 8 and 10, where the sample-to-sample variations tend to be associated with more prolonged upward or downward trends. The same pattern of variation would normally be shown by all animals sampled at the same time, although individual animals varied considerably in the magnitude of these erratic fluctuations. This is apparent from Fig. 4c, where animals 12, 13, and 14 show very similar trends. Animal 15 also shows the same general pattern, but the fluctuation between the 4th and 8th weeks is abnormally great.

Age

Where animals were followed through more than one season. the results—of which those presented in Table 1 are typical—indicated that blood carotenoid levels increase up to about three years of age and thereafter remain relatively constant throughout the adult life of the animal. A few assays only were carried out on the blood of young animals and these are summarised in Table 2. It would seem that, once the animals have access to grass, their blood carotenoid levels, which at birth are very low, rise rapidly, reaching about 50% of adult levels at about 8 to 10 months of age.

Fig. 5 shows the average blood carotenoid and vitamin A alcohol levels for a group of six heifers sampled throughout one season (i.e., from the age of 13 to 21 months). The carotene levels were not as high as those of a comparable group of adult. lactating cows, but, by midsummer, when the heifers were about 18 months old, the two curves had come together and coincided for the rest of the season. The seasonal trends already discussed for adult cows were also apparent in the blood samples from the group of heifers.

TABLE 2. EFFECT OF AGE ON THE LEVELS OF CAROTENOIDS AND VITAMIN A ALCOHOL IN THE BLOOD OF CALVES AND HEIFERS

Age	Breed	No. In Group	Carotenoids (μg/100 ml plasma)		Vitamin A Alcohol (μg/100 ml plasma)
			Carotene	Xanthophyll	
1–3 days	Jersey	3	50	trace	9
3 months	Holstein	3	310	50	11
3 months	Jersey	2	400	55	13
7 months	Jersey	5	630	55	19
8 months	Jersey	1	890	80	—
10 months	Jersey	2	900	75	18
13 months	Predominantly Jersey	6	1240	120	24

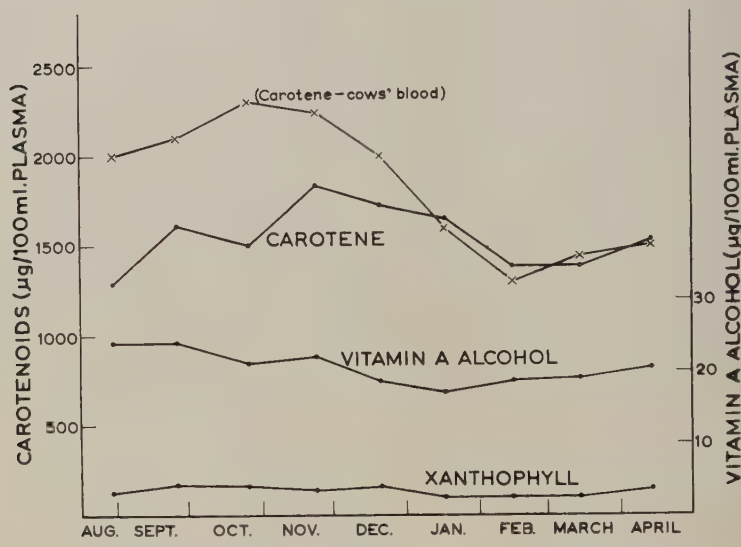


Fig. 5.—Average carotenoid and vitamin A alcohol levels in the blood of a group of six heifers throughout one season. For comparison, the dotted curve shows the levels of carotene in the blood of a comparable group of cows.

Sex

The individual variability already discussed for Jersey cows may be assumed to occur in other breeds, so that little significance can be attached to differences in carotenoid and vitamin A alcohol levels in the blood of the two pedigree Ayrshire cows and bulls. However, as is apparent from Table 3, differences were slight compared with the 3- to 5-fold sex differences between cows and bulls reported by other workers (e.g., Gillam and El Ridi 1937; Sutton and Soldner 1945). It

TABLE 3. Comparison of the Levels of Carotenoids and Vitamin A Alcohol in the Blood of Pedigree Ayrshire Cows and Bulls

Date	Cow/Bull No.	Carotenoids (μg/100 ml plasma)		Vitamin A Alcohol (μg/100 ml plasma)
		Carotene	Xanthophyll	
25th Oct.	Cow 1	890	45	17
	Cow 2	750	50	16
	Bull 1	700	35	16
13th Nov.	Cow 1	1050	55	20
	Bull 1	750	30	18
22nd Nov.	Cow 1	1350	50	17
	Cow 2	900	40	17
	Bull 1	900	30	16
	Bull 2	850	35	15
20th Dec.	Cow 1	1000	35	17
	Cow 2	850	30	15
	Bull 2	780	35	15

would seem, as suggested by Goodwin (1952), that these larger differences are of dietary origin, resulting from the different management of cows and bulls, and that, where both are maintained on pasture throughout the year, differences are no greater than might be anticipated between individual animals of the same breed.

Lactation

Fig. 6 shows the average levels of carotenoids and vitamin A alcohol in the blood of a group of five lactating cows and in the blood of their non-lactating twin-mates. Prior to the end of October shortage of feed necessitated the non-lactating animals being kept on somewhat coarser, more mature pastures than were available for the lactating cows. Although the differences in pasture were slight, they may account for the differences in carotenoid levels over this period, since the carotenoid levels in the blood of the non-lactating cows rose sharply in early November when these animals were transferred to the same pasture as their lactating twin-mates. From this time onwards carotenoid and vitamin A alcohol levels were very similar in the blood of the two groups. The same seasonal trend was apparent in each group.

During the period September to November milk samples were assayed at intervals for carotenoids and vitamin A, and the approximate daily secretions of these substances were calculated for the five lactating cows. These daily secretions were: carotene—4.5 mg; xanthopyll—1.5 mg; vitamin A alcohol—0.2 mg; and vitamin A ester—2.2 mg. The quantities of carotenoids are small relative to the large amounts present in the blood of cows and their uptake by the mammary gland would reduce the blood levels by only about 2% to 2.5% for carotene and about 5% for xanthophyll, amounts which are clearly insignificant in view of the rapid replenishment of blood levels from intestinal absorption

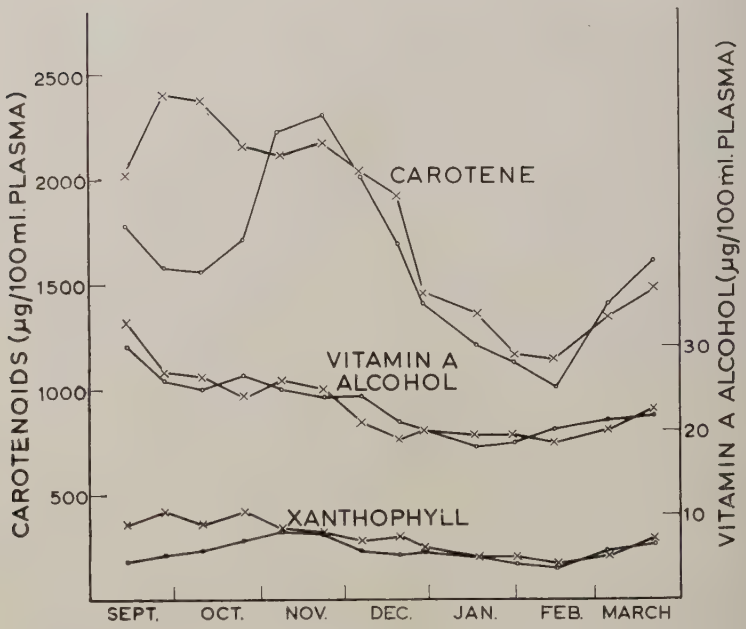


Fig. 6.—Average levels of carotenoids and vitamin A alcohol in the blood of a group of five lactating cows and in the blood of their non-lactating monozygotic twin mates. X — X = lactating; . — . = non-lactating.

(McGillivray 1957d). Similar calculations for vitamin A are not valid, since vitamin A ester, which was not estimated in the blood, is the precursor of most of the vitamin in the milk fat (McGillivray 1957b).

Relationship Between the Levels of Carotenoids in Blood and in Milk Fat

Milk fat carotenoids and vitamin A originate through the uptake by the mammary gland of these substances from the blood. Interest has therefore centred on relationships between the concentrations of these substances in blood and in milk fat. Tarassuk and Regan (1943) report a direct relationship between carotene levels in the blood plasma and in the corresponding milk fat; the numerical value expressed in milligrams per 100 ml plasma and milligrams per 100 g fat should be the same. However, it was apparent that this relationship did not apply under New Zealand conditions of all-the-year pasture feeding. In the case of 50 of the cows in the present survey, milk, as well as blood samples, had been taken for assay on numerous occasions. The concentrations of carotene and xanthophyll in the blood plasma and in the corresponding milk fat samples are plotted in Fig. 7. Where possible, two values have been recorded for each animal, one obtained in spring, when both blood plasma and milk fat levels were high, and another in midsummer, when both were relatively lower. It is apparent that the correlation between blood plasma and milk fat levels is much closer for xanthophyll than

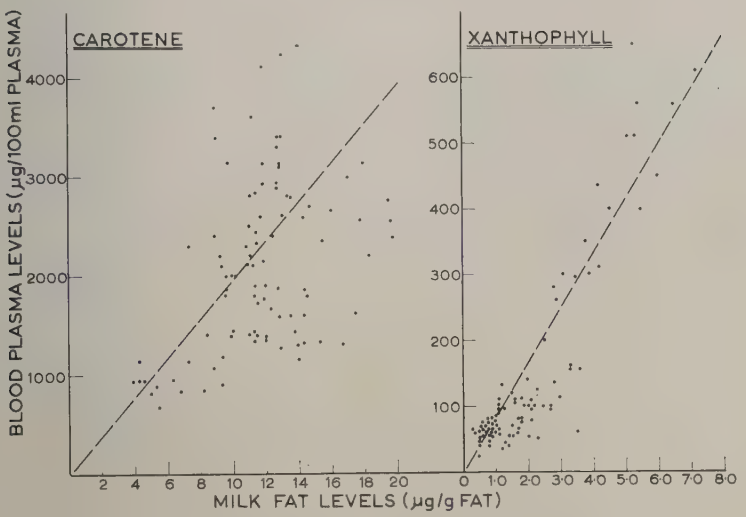


Fig. 7.—Relationships between blood plasma and milk fat levels of carotenoids for 50 individual cows.

for carotene. Assuming that the regression lines should pass through the origin, regression coefficients are

$$F = (+ 0.0051 \pm 0.0018)\ B \text{ for carotene}$$

$$\text{and } F = (+ 0.0122 \pm 0.0005)\ B \text{ for xanthophyll}$$

where F is concentration in milk fat in µg/g and B is concentration in blood in µg/100 ml plasma.

It is clear that, under New Zealand conditions, blood carotene levels are much higher than would be expected from Tarassuk and Regan's relationship; on the average, the relationship, using their units, is that one half of the carotene concentration in the blood plasma, expressed as mg/100 ml plasma, is approximately numerically equal to the concentration in the milk fat expressed in mg/100 g fat. Using these same units, the relationship for xanthophyll concentration in blood plasma and milk fat is more nearly numerically the same.

Conclusions

The main aim of the present communication is: (1) to draw attention to the wide variations in the carotenoid and vitamin A alcohol levels in the blood of individual animals of the same breed under identical conditions of management; and (2) to confirm the seasonal nature of the variations in these constituents already reported for a group of six cows during the 1954–55 season (McGillivray 1957a).

The seasonal trends and the sample-to-sample fluctuations make it clear that factors other than carotene intake have a very marked effect on carotenoid and vitamin A metabolism. These factors are

unassociated with lactation and the fluctuations bear no obvious relationship to climatic conditions. The seasonal trends are in fact very similar in nature and magnitude to those reported by other workers (Barnicoat 1947; McDowell and McDowall 1953; McDowell 1956) in the milk fat of pasture-fed cows in New Zealand. Indeed, these changes in the levels of carotenoids and vitamin A in the milk fat are only a reflection of the changes which occur in the levels of these constituents in the blood of pasture-fed cows. It seems reasonable to assume that the explanation for these variations must lie in changes in the composition of the pasture which influence the absorption of the carotenoids. Despite the very wide range in individual carotenoid and vitamin A levels, all cows appear to respond similarly to these external factors, giving a very uniform picture of the changes in blood levels throughout the season.

The uniformity of monozygotic twin-mates in blood carotenoid and vitamin A alcohol levels is in marked contrast with the variability of unrelated animals and suggests a marked genetic influence over the carotenoid and vitamin A metabolism of animals within the one breed. There seems scope for an investigation of the inheritance of characteristics of this type.

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A STUDY OF THE INTERACTION OF P, K, S, Mg, Mo, AND B FERTILISERS, AND GROUND LIMESTONE, APPLIED TO GRASS-CLOVER PASTURE

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Summary

Results are presented of two multi-factorial field experiments on permanent pasture with six fertilisers and ground limestone. The trials were carried out at Marton Experimental Area and at Invermay Research Station, Mosgiel. Treatments were:—high versus low rates of ground limestone, muriate of potash, and double superphosphate; and some against nil rates of elemental sulphur, magnesium carbonate, borax, and sodium molybdate. All treatments except limestone and sodium molybdate were applied annually by top-dressing.

The main effects and two-factor interactions of these seven treatments on yield, concentration of nutrients in the pasture, herbage composition, and soil quick tests, were determined with considerable accuracy.

Yields were affected by negative LMo, LP, LMg, MgP, and LMoB, and by positive LK, SK, and BP interactions. The negative interactions were the results of two or more treatments acting in the same direction. Heavy liming increased the availability of molybdenum and of phosphorus as measured by plant uptake and dry matter yields. Magnesium carbonate slightly raised soil pH and acted similarly to lime on molybdenum availability. It did not increase phosphorus concentration in plants, but it increased the yield responses on the low phosphorus plots at Marton. Positive interactions generally resulted from simultaneous corrections of deficiencies or toxicities. At Marton yield responses to muriate of potash were increased by an adequate supply of sulphur and by heavy liming. Borax raised yields occasionally on the high phosphate plots, but tended to depress yields on the low phosphate plots.

The concentrations of N, P, K, Ca, Mg, Na, Mn, Mo, B, and Cu in the pasture were influenced by treatments, many interactions taking place.

The results of these trials indicate the importance of balancing fertiliser and lime applications correctly to give maximum returns; they also indicate new lines of investigation in the field of soil fertility and pasture growth.

Introduction

Two field experiments are described which study the interplay of various fertilisers and the value of multi-factorial experiments. Half replicate designs were used, with most high order interactions confounded.

Multi-factorial trials are being used by the Farm Advisory Division of the Department of Agriculture to an increasing extent as a means of examining the effect of trace and major elements on pasture and lucerne growth.

Experimental Details

One experiment was conducted at Marton Experimental Area in the North Island, the other at Invermay Research Station in the South Island near Dunedin.

At Marton the soil type is Marton Loam (N.Z. Soil Bureau 1954), which is derived from a sandy mudstone and a dusting of volcanic ash of andesitic origin.

The soil type at Invermay is Warepa silt loam, a soil derived from acidic drift overlying phonolite.

Both soils are similar in that their subsoil is of heavy texture and slow drainage.

The average annual rainfall is about 42 in. at Marton and about 27 in. at Invermay. Mean annual temperatures (screen) are 53°F at Marton and 50°F at Invermay. At both locations the rainfall is fairly evenly distributed throughout the year. Due to cooler temperatures, winter production of pastures is much lower and often negligible at Invermay. The trial at Invermay was conducted from February 1953 to June 1959, and that at Marton from October 1952 to October 1957.

Treatments

Initial Application

Rates of Initial Application Per Acre

	Substance Applied	Low	High
L:	Carbonate of lime	3 cwt	3 tons
P:	Double superphosphate	½ cwt	2 cwt
K:	Muriate of potash	½ cwt	2 cwt
Mo:	Sodium molybdate	Nil	2½ oz
Mg:	Magnesium carbonate	Nil	3 cwt
S:	Sulphur (elemental)	Nil	56 lb
B:	Borax	Nil	40 lb

In this paper the symbols high L, high P, high K, Mg, S, Mo, and B will be used to denote the treatments receiving high rates of application, or receiving magnesium carbonate, sulphur, sodium molybdate, or borax.

For the sake of simplicity and brevity, differences in response between high and low L, high and low P, and high and low K, will be described as responses to high L, high P, and high K.

Subsequent Applications—Marton

Sodium molybdate was re-applied every second year at the original rate.

Double superphosphate, muriate of potash, and magnesium carbonate were applied annually at the original rates.

Sulphur was applied annually at a reduced rate of 20 lb/acre.

Borax was applied annually at the reduced rate of 10 lb/acre.

Limestone was not re-applied.

Dates of re-application were: 25 November 1953; 5 November 1954; 2 November 1955; and 7 November 1956.

Subsequent Applications—Invermay

Sodium molybdate at 2½ oz per acre was re-applied only in 1955.

From 1957 onwards Ibex brand mono-calcium phosphate replaced TVA double superphosphate, since the TVA product was found to contain 6.5 p.p.m. Mo against 0.1 p.p.m. in Ibex phosphate.

Borax was applied each year, but at a reduced rate of 10 lb/acre from 1955.

Limestone was re-applied at the original rates (3 ton/acre and 3 cwt/acre respectively) in 1955.

All other treatments were applied at the original rates of applications at the following dates: 6 April 1954; 17 March 1955; 1 February 1956; 24 April 1957; 8 May 1958; and 9 April 1959.

Procedure

The trials used a 2^7 factorial half-replicate design, with sub-blocks of eight plots (Finney 1946). Each treatment appeared in half of the 64 plots, giving 32 replications for the estimation of main effects. All high order interactions and some three-factor interactions are confounded in this design, but it was usually possible to arrange confounding so that little information on important interactions was lost.

Plots were 30 ft × 5 ft, and the technique of measurement was "mowing and clippings returned" (Lynch 1947).

Chemical analyses of herbage were carried out on selected mowing cuts. Selected cuts were also dissected into species components to study the effects of treatments on botanical composition.

Soil samples at 0–3 in. and 3–6 in. depth were analysed before the annual top-dressing, using the Truog method for P (Truog 1930) and Morgan's extract for Ca and K (Morgan 1941); pH determinations were made on a 1 : 2½ soil : water suspension by the glass electrode method.

In this paper main effects and interactions are discussed under the following headings:

(1) Soil analyses.
(2) Botanical composition of the sward.
(3) Concentration of nutrients in the herbage.
(4) Yields.

The Effect of Treatments on Soil pH and Soil Quick Test Values

Quick test soil analysis results for the trials before the application of treatments are shown in Table 1.

TABLE 1. Initial Soil Analyses (0–3 in.) Quick Tests

Location	Date Sampled	pH	Ca	K	P
Marton	24 April 1952	5.2 – 5.9	4 – 7	5 – 11	2 – 6
Invermay	30 July 1952	5.9	9	7	3

Interpretation of figures:

Ca — Parts Ca per 20,000 of soil extract (Morgan).
K — Parts K per 200,000 of soil extract (Morgan).
P — Parts P per 50,000,000 of soil extract (Truog).

Table 2 gives more detailed analyses.

TABLE 2. Soil Analyses (0–3 in.), Marton and Invermay, Before Application of Treatments
(mean of all plots)

Location	Cation Exchange Capacity m.e. %	Total Exchangeable Bases m.e. %	Percentage Saturation (%)	Exchangeable Cations m.e.%				p.p.m.	pH
				Ca	Mg	K	Na	Mn	
Marton	18.5	9.4	50	5.7	2.6	0.59	0.44	19	5.4
Invermay	15.9	8.1	51	6.2	1.2	0.31	0.34	7.5	5.9

At Marton soil samples for quick test and pH determinations were taken in spring before each top-dressing. In November of 1953, 1954, and 1955, samples were taken to 3 in. depth. In November of 1956 and 1957 0–1½ in. and 1½–3 in. layers were sampled.

At Invermay top-dressing was carried out in autumn, and soil samples were taken in February or March. All were taken to 0–3 in. depth, but, in 1958, additional samples to 0–1½ in. depth were collected. Changes in soil test values due to treatments are shown in Table 3.

Discussion.—Three tons of limestone per acre (96.9% $CaCO_3$) raised the soil pH by about one unit in the top 3 in. of soil. Owing to the application of an additional 3 tons of limestone per acre at Invermay in March 1955, the soil analyses taken from November 1955 onward compare the effect of 3 tons of limestone against 3 cwt per acre at Marton, and of 6 tons of limestone against 6 cwt per acre at Invermay. The extra 3 tons at Invermay have not resulted in a marked additional rise in soil pH.

The high L treatment depressed the soil K test value, particularly at Marton. In pasture measurement trials, in which clippings are returned whenever possible, a decline of the soil test for potassium has been associated with high-yielding plots. This has been explained by the fact that, when it is necessary to discard clippings during time of rapid growth, the more highly-producing plots suffer a greater loss of potassium in the form of discarded clippings. Since, at Marton, the yield increases due to high L and those due to high P are similar, and,

TABLE 3. QUICK TEST SOIL ANALYSES, 0–3 in., RESPONSES TO TREATMENTS, MARTON AND INVERMAY

Mean Values On "Low" Or "Nil" Treatments					Treatments	Responses To Treatments *Changes From "Low" Or "Nil" Treatment					
						pH		K Test		P Test	
Date Sampled	Location	pH On Low L Plots	K On Low K Plots	P On Low P Plots		Mar†	Inv†	Mar	Inv	Mar	Inv
November 1953	Marton	5.5	7.0	3.8	High L	1.2	0.8	—0.9	—	—	—
					High P	—		—	—0.6	1.3	1.5
February 1954	Invermay	6.1	4.4	2.9	High K	—	—	—	1.8	—	—
					Mg	0.1	—	—	—	—	—
November 1954	Marton	5.8	3.3	2.7	High L	1.1	0.9	—1.1	—	1.7	1.2
					High P	—	—	—	—	3.7	3.5
February 1955	Invermay	6.0	5.3	4.2	High K	—	—	0.9	2.0	—	—
					Mg	0.2	—	—	—	—	—
November 1955	Marton	5.7	3.3	4.5	High L	1.1	1.1	—0.8	—	2.9	1.6
					High P	—	—	—	—	5.9	4.3
					High K	—	—	0.9	2.0	—	—
February 1956	Invermay	5.9	5.2	4.2	Mg	0.2	—	—	—	—	—
					S		—	—0.6	—	—	—
November 1956	Marton, average of 0–1½ in. and 1½–3 in. samples				High L	1.0	1.2	—1.2	—	2.3	2.7
		5.7	4.8	4.5	High P	—	—	—	—	6.5	4.5
March 1957	Invermay	6.0	6.7	6.2	High K	—	—	—1.2	2.1	—	—
					Mg	0.2	0.1	—	—	1.2	—
November 1957	Marton, average of 0–1½ in. and 1½–3 in. samples				High L	0.8	0.9	—1.1	—1.0	2.4	1.4
		5.6	3.4	4.4	High P	—	—	—0.4	—	6.4	4.6
March 1958	Invermay	5.8	5.6	5.6	High K	—	—	0.8	1.5	—	—
					Mg	0.2	—	—	—	1.5	—
					S	—	—	—	—	—1.3	—

* Only differences statistically significant at the 1% level are shown.

† Mar = Marton. Inv = Invermay.

since the high L treatment has led to a more marked depression of the K soil test value, it is likely that other factors are also involved.

Heavy liming led to an increase in P soil test values, an increase which was reflected in the greater concentration of P in the herbage grown on the high-lime plots.

The main effect of the high phosphate treatments on the Truog P soil test levels was very similar at both localities.

The main effect of the high potash treatments on the K soil test level was more marked at Invermay. This difference in the effect of the high K treatments on soil test at the two sites is thought to be partly due to the greater quantity of clippings discarded at Marton.

TABLE 4. Consistent Interactions Affecting pH and Truog Soil P Test at Marton and Invermay (0–3 in.)

Type Of Interaction	Data Affected	Time Interaction Occurred
Negative L Mg Marton	pH	1954**, 1955**, 1956**, 1957**, (all depths of sampling).
Negative L Mg Invermay	pH	1956*, 1957**, 1958* (0–1½ in.), 1959*
Positive L P Marton	P	1956** (1½–3 in.), 1957* (0–1½ in.), 1957** (1½–3 in.).
Positive L P Invermay	P	1955**, 1956**, 1957**, 1958** (0–1½in.).

* Statistically significant at the 5% level.
** Statistically significant at the 1% level.

Table 4 shows that there were two consistent interactions involving rates of limestone. In the negative L Mg interaction on soil pH magnesium carbonate raised soil pH more markedly in the low lime plots. Whenever the positive LP interaction on the Truog soil P test occurred the high rate of double superphosphate increased the Truog test level more markedly on the high lime, than on the low lime, plots.

The negative LMg interaction on pH indicates that in these two trials it is more difficult to obtain an upward change of soil pH at about neutrality than at about pH 6.0. This evidence is strengthened by the apparently negligible effect of the repeat application of 3 tons of limestone per acre on soil pH in Invermay.

The Effect of Treatments on Pasture Composition

The effect of treatments on pasture composition was determined by dissecting the cut herbage into individual species or groups of species (Brown 1954).

Tables 5 and 6 summarise the average percentages of species in the cut herbage at the dates indicated. The dissections were more detailed on some occasions than on others.

TABLE 5. MEAN PASTURE COMPOSITION IN THE CUT HERBAGE, DRY MATTER BASIS

MARTON

Date of Cut	% Lolium Perenne	% Poa Species *	% Dactylis Glomerata	% Other Grasses	% Total Grasses	% Trifolium Repens
16 September 1953	23	34	17	—	74	22
7 September 1954	31	15	23	—	69	25
4 October 1955	49	10	18	—	77	13
19 November 1956	10	27	35	9	72	13
9 January 1957	7	7	25	—	39	42
25 March 1957	6	—	29	—	35	44
23 October 1957	18	28	21	9	76	18

* Poa trivialis and Poa annua.

At Marton in the last two years the proportion of perennial rye-grass (*Lolium perenne*) decreased appreciably and brown top (*Agrostis tenuis*) became very prominent on the low-producing plots.

TABLE 6. MEAN PASTURE COMPOSITION IN THE CUT HERBAGE, DRY MATTER BASIS

INVERMAY

Date Sampled	% Lolium Perenne	% Dactylis Glomerata	% Other Grasses	% Total Grasses	% Trifolium Repens	% Trifolium Pratense	% Weeds
16 October 1953				57	21	22	*Tr
6 April 1954				50	20	29	Tr
8 March 1955	22	30	5	57	34	6	2 (1% dead matter)
11 May 1956				69	25	5	1
6 October 1956				73	22	4	1
18 February 1957				44	48	7	1
25 March 1958	31	9	15	55	39	3	Tr (3% dead matter)
9 December 1958	48	6	14	68	29	3	Tr
13 February 1959	43	10	5	58	29	4	Tr (9% dead matter)

* Tr = trace or less than 1% present.

Tables 7 and 8 show the increases or decreases in percentage of species due to treatments, at Invermay and Marton.

The Main Effect of Heavy Liming. Although the high L treatment raised the productivity of the sward to about the same degree in both trials (about 10% over low L), its effect on pasture composition was very different.

TABLE 7. The Main Effect of Lime and Molybdenum Treatments on Percentage Pasture Composition, and the Occurrence of LMo and LMoB Interactions, Invermay

Date of Cut	Main Effects						Interaction			Mean Composition %		
	High Lime			Molybdenum			LMo					
	Grass	Trifolium Pratense	Trifolium Repens	Grass	Trifolium Pratense	Trifolium Repens	Grass	Trifolium Pratense	Trifolium Repens	Grass	Trifolium Pratense	Trifolium Repens
16 October 1953	7.5**	—5.4**	—	—	—3.4*	4.7*		+ **	(LMoB**)	57	22	21
6 April 1954	6.8**	—7.6**	—	—	—4.1**	2.9**				50	29	20
9 March 1955	(R) –3.4**	—	—	(R) –3.4**	—2.0*	8.9**	(R) + *	+ **	— *	57	6	34
	(C) 5.2**											
28 February 1956	—	—	3.3*	—	—3.9**	3.2**			— *	57	7	17
11 May 1956	—	—	3.2*			3.5*				69	5	25
6 October 1956	3.9**	—	—3.0**	-	—1.6*	2.9**	(LMoB*)		(LMoB**)	73	4	22
18 February 1957	—	—	3.9*	—	—2.1*	9.1**	+ **		— **	44	7	48
10 October 1957	—	—	—	—	—	3.3*				75	3	22
25 March 1958	(R) 6.1*	—	—	—						55	3	39
	(C) –1.4*	—	—	—	—	4.1*						
	(O) –6.3*	—	—									
9 December 1958	(R) 7.3**			(R) 5.7*			(O) + **			68	3	29
	(C) –1.9*				—2.6*	5.9**	(C) (LMoB*)					
	(O) –8.1**	—2.2*	4.8*	(O) –6.9**								
13 February 1959	(O) –3.8**	—2.6**	6.1**	(O) –2.0*	—2.6*			+ *		58	4	29
13 April 1959	(O) –4.5**	—2.0**	9.1**	(O) –3.3**	—1.7**	4.6**		+ *	— **	65	3	24

* Significant at 5% level.
** Significant at 1% level.

R = Lolium perenne.
O = Other grasses.
C = Dactylis glomerata.

TABLE 8. The Main Effects of Treatments on Percentage Pasture Composition at Marton

	High Lime		High Phosphate	High Potash		Sulphur		
Date of Cut	Poa Spp.	Trifolium Repens	Lolium Perenne	Poa Spp.	Trifolium Repens	Lolium Perenne	Poa Spp	Trifolium Repens
16 September 1953	12.7**	8.3**	—	—	—	—	—	—
16 September 1954	7.5**	—6.8**	—	—	4.4**	—	—	—
4 October 1955	3.2**	—	—	—2.1*	3.5**	—	—	—
30 October 1956	—	—1.5*	—	—	5.4**	—	—	—
19 November 1956	11.4**	—	—	—	5.5**	—	—	—
9 January 1957	2.5**	—	1.7*	—2.1**	10.1**	—	—	7.5**
25 March 1957	—	—	—	—	8.8**	1.7*	—	7.0*
23 October 1957	8.6**	—	—	—	3.6*	4.3*	2.9*	—

* Significant at 5% level.
** Significant at 1% level.

In the Marton Trial (Table 8), the high L treatment lowered the proportion of *Trifolium repens* and raised the proportion of Poa species. It had no effect on the proportion of *Lolium perenne*.

At Invermay (Table 7), high L increased the proportion of the species demanding high fertility, namely *Lolium perenne* and *Trifolium repens*, but it decreased the proportion of species which tolerate somewhat lower fertility, namely *Dactylis glomerata*, *Trifolium pratense*, and weed grasses.

The difference in the effect of high L in the two localities is probably due to molybdenum. At Invermay heavy liming increased available molybdenum from deficient to adequate levels, thereby stimulating the growth of *Trifolium repens*, whereas at Marton soil molybdenum was in fairly adequate supply on the low lime plots.

The Main Effect of High Phosphate. At Marton the high P treatment had no appreciable main effect on the composition of the sward, but it took part in interactions which are discussed later.

At Invermay (not shown in Tables) the high P treatment increased the proportion of *Trifolium repens* in the sward, but to a lesser degree than heavy liming. It markedly decreased the proportion of *Trifolium pratense* and it increased the proportion of *Lolium perenne*.

The Main Effect of High Potash. At Invermay the high K treatment began to improve herbage yields slightly in occasional seasons from 1956 onwards. At Marton it increased the level of herbage yields quite markedly after the first year, from 1953 onwards. In both trials high K increased the proportion of *Trifolium repens* in the sward, at Marton from 1954 onwards, at Invermay (not shown in Tables) from 1956 onwards. It had little effect on the proportion of other species in the sward.

The Main Effects of Magnesium Carbonate and Borax. Both these treatments produced few statistically significant main effects on pasture composition.

The Main Effects of Sodium Molybdate. At Invermay, where a moderately good growth response to molybdenum was obtained (12% on low lime plots, 3% on high lime plots), sodium molybdate materially increased the proportion of *Trifolium repens*, and depressed the proportion of *Trifolium pratense*, more consistently than any other treatment. Although the proportion of *Trifolium pratense* was reduced, this species showed a response to molybdenum in terms of absolute dry matter yields. Molybdenum differed in its effect from high L by stimulating clover species more quickly and by having little effect on the proportion of grass species, except in the last year.

Interaction Effects on Pasture Composition

The LMo Interaction. At Invermay there was a consistent tendency for a negative LMo interaction on *Trifolium repens* and a positive LMo interaction on *Trifolium pratense* and on grasses (see Table 7).

The molybdenum treatment, by increasing the competitive strength of *Trifolium repens*, markedly reduced the proportion of grasses

on the low lime plots. The high L treatment led to similar results on the plots without Mo, although there is evidence that it increased the growth of grasses on plots both with and without Mo.

At Marton no consistent LMo interaction on pasture composition took place.

The LMg Interaction. At Invermay a consistently occurring LMg interaction (not recorded in the Tables) was positive on the grasses and *Trifolium pratense,* and negative on *Trifolium repens.*

Magnesium carbonate increased the percentage of *Trifolium repens* on the low lime plots but not on the high lime plots. High lime increased the proportion of *Trifolium repens* on the plots without Mg but not on those with Mg.

The LMg interactions thus ran parallel to the LMo interactions.

The LBMo Interaction. This unexpected interaction was statistically significant on eight occasions (four times at both Marton and Invermay) on herbage composition, mainly in spring and early summer. At Marton this interaction also affected yields in the totals for two years.

The effect of the LBMo interactions on pasture composition can be expressed as follows. The LMo interaction on grasses was positive without B, but negative with B. The opposite occurred twice on *Trifolium repens* at Invermay. The LMo interaction, which was normally negative, became positive on the plots treated with borax, but remained negative on the plots without borax.

Discussion on Herbage Dissection Results and Yields of Herbage. The effect of treatments on herbage composition, and the influence of changes in herbage composition on herbage yield, were predictable at Invermay but not at Marton.

At Invermay the treatments which stimulated yields generally raised the proportion of *Trifolium repens* and *Lolium perenne* in cut sward. This did not hold to the same extent at Marton. Only the high K treatment consistently raised the percentage proportion of *Trifolium repens* and yields. The high L treatment, on the other hand, increased the percentage of Poa species and at times depressed the proportion of *Trifolium repens.*

The Effect of Treatments on the Concentration of Nutrients in the Cut Herbage

Table 10 summarises the main effects of treatments on the concentration of N, P, K, Na, Mg, Ca, Mn, and Mo in the cut herbage at Marton.

Due to the considerable amount of work involved, only the October 1957 sample was dissected into clovers and grasses, the other five samples being mixed herbage.

The large replication achieved by the factorial layout makes the trial very accurate, so that small differences in concentration are

commonly statistically significant. However, the interpretation of these small changes in the concentration of many nutrients is complicated by the fact that the proportion of clovers and grasses in the sward was altered by some treatments.

Table 9 summarises the main effects of treatments on the concentration of minerals in the cut herbage at Invermay. Only four mixed pasture samples were analysed from the Invermay trial. Boron and copper concentrations were also determined in one cut.

In the following discussion the main effects and the treatment interactions are dealt with together, since many significant interactions were found.

The Effect of Treatments on the Nitrogen Concentration of the Herbage. The interpretation of the main effects on N concentration in the herbage is complicated by several factors. Clovers usually contain considerably more N (on a dry matter basis) than grasses. Treatments which increase the proportion of clovers in the sward are therefore likely to increase the concentration of N in the mixed herbage. On the other hand, it seems that, in grasses, moderate variations in the supply of soil nitrogen may be reflected in rate of growth rather than in N concentration of the herbage. In these trials the main effects of treatments on nitrogen concentration in the herbage seldom exceeded 5%. Many of these slight changes can be explained by variations in the species composition of the pasture. Interaction effects on the concentration of nitrogen were few.

For these reasons consideration is given only to the treatment effects on N concentration in the herbage which did not simultaneously affect herbage composition.

Invermay

In the cut of April 1958, high L increased the N concentration without affecting, to any extent, the proportion of grasses and clovers in the sward. Heavy liming increased the supply of available nitrogen, partly due to its effect on releasing soil molybdenum, but possibly also by stimulating the activity of some micro-organisms in the soil.

A highly significant BP positive interaction occurred in the same cut of April 1958. Borax depressed N concentration slightly in the low P plots but increased the N concentration on the high P plots. This interaction shows the same trend as that obtained at Marton and agrees with the positive BP interaction occasionally obtained on yields which occurred at both trials.

Marton

The mean value of N concentration in the mixed herbage was remarkably uniform and the main effects of treatments were very slight. Table 11 shows the interactions significant at the 1% level and those which were reasonably consistent at the 5% level of significance.

In the cut of December 1953 molybdenum increased the nitrogen concentration in the herbage in the absence, but not in the presence, of magnesium carbonate.

TABLE 9. SIGNIFICANT MAIN EFFECTS OF TREATMENTS ON THE CONCENTRATION OF NUTRIENTS IN MIXED HERBAGE, DRY MATTER

INVERMAY

Date Sampled and Treatment	Percentage Change in Chemical Concentrations									
	P	K	Na	Mg	Ca	N	Mo	Mn	B	Cu
December 1953										
High L		—5**		—6**		—5**	29**	—9**		N.A.
High P	21**	5**	23**	18**	28**	16**				
High K	—4**	7**	—15**				—12*			
Mg					—7*					
S							—13*	5*	12*	
Mo			11**				587**		—14**	
B		—3**							33**	
Mean Value	0.28%	2.58%	0.25%	0.28%	1.00%	2.6%	0.86 p.p.m.	158 p.p.m.	10.9 p.p.m.	
March 1955										
High L	7**	3*		—7**	8**		N.A.	—37**	N.A.	N.A.
High P	33**		14**				N.A.		N.A.	
High K		6**	—23**				N.A.	—9*	N.A.	
Mg				5*			N.A.	—9*	N.A.	
S							N.A.	10*	N.A.	
Mo	—7**		15**			8**	N.A.	—16**	N.A.	
B							N.A.		N.A.	
Mean Value	0.37%	3.5%	0.24%	0.26%	0.84%	4.1%	N.A.	144 p.p.m.	N.A.	

May 1956										
High L	10**	6**		—7**	14**	5**	156**	—63**	N.A.	N.A.
High P	26**		18**						N.A.	
High K		10**	—37**	—7**	—3*				N.A.	
Mg	—4**			26**	—7**		51**	—23**	N.A.	
S							—31**		N.A.	
Mo	—3**						274**	—21**	N.A.	
B				—6*					N.A.	
Mean Value	0.38%	2.9%	0.16%	0.30%	0.90%	3.9%	0.64 p.p.m.	314 p.p.m.	N.A.	
April 1958										
High L	9**		19**	—7**	11**	5**	56**	—54**	N.A.	—6*
High P	67**	—7**	26**	8**						—8**
High K		27**	—49**	—7*	—6**			—24**		
Mg				8**			—22*			
S						5**	126**	—24**		
Mo		—5**	19**							
B	7*									
Mean Value	0.34%	2.62%	0.35%	0.26%	1.08%	3.83%	0.44 p.p.m.	145 p.p.m.		8.3 p.p.m.

N.A. = Not analysed. * Sig. at 5% level. ** Sig. at 1% level.

The differences in nutrient concentration of the herbage between the high rate of application and the low or nil rate is expressed as percentage change from the low or nil rate. For instance, in the cut of December 1953, the potassium concentration was 5% lower on the high L plots than on the low L plots and 5% higher on the high P plots than on the low P plots.

TABLE 10. SIGNIFICANT MAIN EFFECTS OF TREATMENTS ON THE CONCENTRATION OF NUTRIENTS IN THE HERBAGE, DRY MATTER

MARTON

Date Sampled	Treatment	Percentage Change in Chemical Concentration							
		P	K	Na	Mg	Ca	N	Mn	Mo
December 1953 Mixed Pasture	High L	8**			—10**	10**	—4**	—34**	164**
	High P	14**					2*		
	High K		28**	—18**			4**		—12*
	Mg	—8**			11**	—5*			
	S								—10*
	Mo								106**
	Mean Value	0.38%	2.14%	0.40%	0.40%	0.77%	3.8%	200 p.p.m.	1.25 p.p.m.
January 1955 Mixed Pasture	High L	18**	—6*	24**	—12**	23**	4**	—51**	58**
	High P	33**	—8**	22**	6*	7**	3*	—17**	
	High K	—11**	58**	—34**	—8**				—18*
	Mg				14**	—4*	3**	—22**	22*
	S	—4*							—40**
	Mo								671**
	Mean Value	0.345%	1.68%	0.65%	0.37%	0.90%	3.86%	178 p.p.m.	1.78 p.p.m.
October 1955 Mixed Pasture	High L	6**			—10**	22**		—51**	
	High P	21**	—12**	11**				—9*	
	High K	—2*	30**	—22**					N.A.
	Mg				10**	—9**		—20*	
	S	—2*							
	Mean Value	0.448%	1.86%	0.81%	0.29%	0.54%	3.81%	161 p.p.m.	

January 1956 Mixed Pasture	High L	8**	—10**		—10**	15**		—54**	78**
	High P	27**							
	High K	—9**	45**	—45**	—8**			12*	—21**
	Mg				14**	—6*		—20**	27**
	S	—3*							—44**
	Mo								171**
	Mean Value	0.38%	1.98%	0.42%	0.42%	0.91%	3.56%	232 p.p.m.	1.3 p.p.m.
November 1956 Mixed Pasture	High L			—15**	—2**	23**	—3**	—51**	
	High P	16**						—10*	
	High K		38**	—43**	—7**	—5**		13**	N.A.
	Mg				37**	—12**		—23**	
	S						2*		
	Mean Value	0.53%	2.97%	0.35%	0.34%	0.58%	3.8%	192 p.p.m.	
October 1957 Grasses Only	High L	5**			—13**	28**		—53**	
	High P	38**	—13**	31**				—13**	
	High K		46**	—33**	—11**	—12**			N.A.
	Mg				19**	—9**		—33**	
	S	—6**	—10**	20**					
	Mean Value	0.45%	2.14%	0.63%	0.28%	0.37%	3.46%	153 p.p.m.	
October 1957 Clovers Only	High L	6*	—12**		—16**	17**	—1*	—40**	
	High P	26**	—15**	18**		5*	2*		
	High K	—4*	51**	—36**	—6*		—3**		N.A.
	Mg		—7*		21**	—9**		—29**	
	S	—7**	—13**	12**	—5*	—6**	3**	—12*	
	Mean Value	0.44%	1.54%	0.56%	0.35%	0.95%	5.5%	76 p.p.m.	

N.A.= Not Analysed. * Sig. at 5% level. ** Sig. at 1% level.

TABLE 11. Treatment Interaction Effects on N Concentration of Herbage, Marton

	Date of Cut		
	December 1953	January 1955	January 1956
Type of Interaction	MgMo**	LP negative **	MoL negative **
	Negative	BP positive *	BP positive *

* Significant at 5% level
** Significant at 1% level.

In the cut of January 1955 the high L treatment appreciably increased the N concentration of the low phosphate plots, but not of the high phosphate plots. High P increased the N concentration on the low L plots only.

In the cut of January 1956 the N concentration was increased by high L on the plots without Mo, but Mo depressed the N concentration of the herbage on the high L plots.

The Effect of Treatments on the Phosphorus Concentration in the Herbage. The phosphorus concentration is usually similar in grasses demanding high fertility and in *Trifolium repens,* although there may be differences in P concentration between low-fertility- and high-fertility-demanding grass species. This fact assists in the interpretation of results to be discussed.

Invermay

The mean value of P concentration, and, particularly, the P concentration on the low P plots (0.26% in dry matter), is low in the cut of December 1953 and it is further lowered very slightly on the high K treatments.

The two consistent treatment main effects on P concentration are (apart from the high P treatment) those of high L and Mo. The former increased the P concentration in all but the 1953 sampling, while the latter caused a slight fall in the cuts of March 1955 and May 1956. The action of molybdenum could be interpreted as a dilution effect due to higher yields. On the other hand, Hewitt (1957) has shown that molybdenum deficiency may affect the total P concentration and the proportion of various fractions of P in cauliflowers.

The high L treatment increased herbage yield as well as the concentration of P. The magnesium carbonate treatment decreased the concentration of P in the cut of May 1956. In the cut of December 1953 it entered into a highly significant interaction with molybdenum. Magnesium carbonate depressed the P concentration in the absence of molybdenum but increased it in the presence of molybdenum.

Marton

The mean value of the P concentration in the herbage is much higher in the spring, than in the summer, cuts (December and January).

The fluctuation in P content of pastures at Marton was also observed by Doak (1933). He found high values in September and early October, followed by a gradual fall, reaching a minimum in the period from January to mid-April.

The high L treatment increased the P concentration in the herbage appreciably and consistently. This was in agreement with results at Invermay and would indicate that, on these soil types—both yellow-grey earths—heavy liming may have a beneficial effect on the uptake of phosphorus. It is not known whether this effect is confined to better utilisation of applied fertiliser phosphate or of soil phosphates, or whether the phosphorus returned in clippings might be broken down and mineralised more rapidly when in contact with a well-limed soil. At this stage it would be unwise to conclude that liming raises the concentration of phosphorus in grazed pastures.

The high K treatment lowered the concentration of P in the herbage. This may be partly the result of a dilution effect due to higher yields, a view which is consistent with the fact that, in the cut of October 1957, the P concentration was lowered by high K in the clovers but not in the grasses. Potassium fertilisation stimulated mainly clover growth.

Magnesium carbonate lowered the P concentration at the 1% level of significance in the cut of December 1953. It had the same action at Invermay in the cut of May 1956. Later chemical analyses, however, did not reveal any such trend. On the contrary, on two occasions magnesium carbonate counteracted a fall in P concentration on the plots treated with borax (see Table 12).

Sulphur on four occasions lowered the concentration of P in the herbage. This might have been due to a dilution effect.

The remaining treatments did not exert main effects on the concentration of P in the herbage.

However, several consistent or highly significant interactions did occur. These are summarised in Table 12.

TABLE 12. Treatment Interaction Effects on the P Concentration of Herbage, Marton

Treatments	Date Of Cut			
	January 1955	October 1955	January 1956	November 1956
Type of Interaction	LK** negative KP* negative	LP** negative MgB** positive LMo* positive	KP* negative LMo** positive	KP* negative
	October 1957 grasses			
	LP** negative MgB* positive			

* Significant at 5% level.
** Significant at 1% level.

In the negative KP interaction the high K treatment lowered the P concentration in the herbage much more on the low P plots than on the high P plots, indicating that the depressing effect of the high K treatment on the concentration of P in the herbage was not entirely due to a dilution effect. In the negative LK interaction of January 1955 the P concentration in the herbage was much more markedly raised by high L on the low K plots than on the high K plots. There was no significant LK interaction on yields at that time, so that a dilution effect cannot be put forward as an explanation.

In the MgB positive interaction occurring in the cuts of October 1955 and October 1957 (grasses), borax depressed the concentration of phosphorus (highly significantly in October 1955) on the plots not receiving magnesium carbonate, but had no effect on P concentration in the plots receiving magnesium carbonate.

In the negative LP interaction high L increased the P concentration in the herbage appreciably on the low P plots but not on the high P plots. A parallel negative interaction was also obtained on herbage yields, indicating a sparing effect of lime on phosphorus applied in sub-optimal amounts.

In the positive LMo interaction in the cut of January 1956 high L barely increased the P concentration in the herbage on the plots without Mo but markedly increased it on the molybdenum-treated plots.

The Effect of Treatments on the Potassium Concentration in the Herbage

Invermay. It appears likely that increases in the proportion of clovers in the sward led to decreases in the concentration of potassium in the mixed herbage. Changes in K concentrations which might have been the result of simultaneous changes in species composition are therefore not discussed.

The high L treatment caused a decrease in K concentration in the mixed herbage at the first sampling, followed by an increase in the two subsequent samplings. It is difficult to explain this effect.

Surprisingly, the responses of K concentration to the higher rate of potash became appreciable only in the last cut to be analysed, by which time the K concentration on the low K plots had fallen to 2.3%.

The high phosphate treatment increased the K concentration in the December 1953 cut, whereas, in the April 1958 cut, it depressed the K concentration in agreement with results obtained fairly consistently at Marton.

Marton. The mean value of the K concentration fluctuated considerably. It was particularly high in the mixed herbage in November 1956—2.5% in the low K plots and 3.4% on the high K plots—yet the high K treatment produced a larger growth response than any other treatment at that time.

In October 1957, when the trial was discontinued, the K concentrations in the grasses and clovers were as follows:

K Concentration in Herbage, % Dry Matter, Marton, October 1957

	Low K	High K	Increase
Grasses	1.74	2.54	0.80
Clovers	1.23	1.86	0.63

As the critical K concentration in *Trifolium repens* growing in a clover-grass pasture is approximately 1.8% (McNaught 1958), it is likely that, even in the high K plots, the supply of available potassium to clovers was only marginal some 11 months after the last top-dressing with 2 cwt KCL/acre.

The high L treatment decreased the K concentration in the clover in the cut of October 1957, and in the mixed herbage in the cuts of January 1955 and January 1956, but not in the spring cuts (when clover constituted a lower proportion of the sward). It is possible that the high L treatment, by increasing the proportion of Poa species in the sward, and hence the proportion of grasses as a whole, led to a reduction of the potassium available to the associated clovers on a soil which contained only marginal supplies of available potassium.

The high P treatment depressed the concentration of K in the herbage in four out of a total of seven sets of analyses. This treatment had a similar effect on grasses and clovers in the cut of October 1957 when it was too large to be explained entirely by a dilution effect due to stimulated growth rate.

The sulphur treatment depressed the K concentration only in the cut of October 1957 in clovers and grasses. Its action may be mainly due to a dilution effect.

The magnesium carbonate, borax, and molybdenum treatments had little or no effect on the concentration of K in the herbage.

There were very few treatment interaction effects on the K concentration of herbage. The SK negative interaction on K concentration in both clovers and grasses was the only one significant at the 1% level. Sulphur decreased the concentration of K in both clovers and grasses quite markedly (clovers—18%, grasses—16%) on the high K plots. It reduced K to a negligible extent on the low K plots. Since the growth response to sulphur was at that time in the vicinity of only 6%, it can be assumed that the cumulative effect of several annual dressings of elemental sulphur had reduced the available potassium in the soil under the conditions of this trial. It is also possible that the slightly heavier yields on the sulphur plots led to relatively greater losses of potassium from those plots when herbage had to be discarded.

The Effect of Treatments on the Sodium Concentration in the Cut Herbage. In general, treatments which increased the concentration of K in the herbage lowered the concentration of Na; conversely, treatments which lowered the concentration of K increased the concentration of

Na. The high P treatment, however, consistently increased the concentration of Na in the herbage, even at times when it had little or no effect on the concentration of K. Molybdenum acted similarly to high P at Invermay. It increased appreciably the concentration of Na in the herbage in the three analyses of December 1953, March 1955, and March 1958. The amount of sodium added in the sodium molybdate was insignificant. This effect is probably associated with the greater proportion of clovers on the molybdenum-treated plots.

The Effect of Treatments on the Concentration of Magnesium in the Herbage. At both Invermay and Marton the Mg concentration of the herbage was high. Since clovers commonly, but not always, contain a much higher concentration of Mg than grasses, the interpretation of treatment effects on Mg concentration is somewhat hampered by the fact that only one series of samples was dissected into clover and grasses.

Invermay

The high L treatment consistently, but relatively slightly, lowered the concentration of Mg in the herbage. The high P treatment increased the Mg concentration in the cuts of December 1953 and April 1958. The high K treatment reduced the Mg concentration to a surprisingly small degree in the cuts of May 1956 and March 1958. It is likely that the latter effects were due to ion antagonism.

The effect of annual dressings of 3 cwt magnesium carbonate per acre on the magnesium content of the pasture appears to have been relatively small, possibly a reflection of the already high level of available magnesium in the soil. It had no effect 10 months after the first application, and only a very slight effect in the cut of March 1955, some 12 months after the second application. However, it exerted a more marked effect some 14 months after the third application. By this time 9 cwt of magnesium carbonate had been applied.

Only one highly significant treatment interaction affected Mg concentrations in the herbage: the LPB interaction in the cut of March 1958. On the low L plots borax depressed the Mg concentration quite considerably on the low P plots, but not on the high P plots.

Marton

The main effects were similar to those obtained at Invermay. The high L treatment consistently reduced the Mg concentration in the herbage. The high P treatment increased the Mg concentration once, in the cut of January 1955. The high potassium treatment, although it increased the proportion of clovers in the sward, had a slight, but fairly consistent, depressing effect on the concentration of Mg. There were very few treatment interactions on the Mg concentration in the herbage.

The Effect of Treatments on the Calcium Concentration in the Cut Herbage

Invermay. The lack of chemical analyses on dissected samples weakens the results of Ca analyses considerably because clovers generally contain much more calcium than grasses.

Nevertheless, a shift in pasture species barely suffices to explain the quite considerable increase in the Ca concentration in the cut of December 1953 due to the high P treatment. In the same cut the lack of increase in the Ca concentration in the herbage due to the high L treatment can be partly explained by the increase in the proportion of grasses and the decrease in the proportion of *Trifolium pratense*. In the cuts of March 1955, May 1956, and March 1958, the high L treatment slightly increased the concentration of Ca in the mixed herbage. At these dates the high L treatment had relatively little effect on the herbage composition of the sward.

Magnesium carbonate reduced the Ca concentration in the herbage on two occasions.

The high K treatment slightly decreased the concentration of Ca in the herbage in the two last cuts analysed—those of May 1956 and April 1958.

The only highly significant interaction affecting the Ca concentration in the herbage occurred in the cut of April 1958. Sulphur depressed the Ca concentration slightly in the low K plots but increased it on the high K plots.

Marton. The mean value of the Ca concentration in the herbage fluctuated considerably; it was lower in spring than in summer, probably mainly due to the greater proportion of clovers in the sward during summer. The treatment effects were very similar to those obtained at Invermay.

Magnesium carbonate consistently depressed the concentration of Ca in the herbage; probably this was due to an ion balance effect.

The high P treatment twice increased the concentration of Ca, once in the mixed herbage and once in clovers (but not in the grasses). As at Invermay, there is evidence that the high K treatment slightly depressed the Ca concentration in the herbage. This occurred only towards the end of the trial in the January 1956 and November 1956 cuts of mixed herbage, and in the cut of October 1957 in grasses.

Sulphur slightly, but highly significantly, decreased the Ca concentration in clovers in the October 1957 cut. This could have been due to a dilution effect associated with growth responses to the sulphur treatment.

The Effect of Treatments on the Manganese Concentration in the Herbage. The manganese concentration of the herbage may be markedly affected by the proportion of clovers to grasses in the sward. The concentrations of manganese in grasses is commonly twice as high as in clovers.

Invermay

The effect of the high L treatment on the Mn concentration of the herbage was surprisingly small in the first cut, which was taken 10 months after 3 tons of lime per acre had been applied. It is very likely that this small apparent effect was not entirely due to a slow action of

the limestone but was counteracted by a shift in the proportion of species in the sward. The proportion of *Trifolium pratense* was reduced, and the proportion of grasses was increased, by the high L treatment at that time. In later cuts the high L treatment appreciably reduced the Mn concentration.

The magnesium carbonate treatment also decreased the concentration of Mn in the herbage, an effect which is best illustrated by considering the positive LMg interaction occurring in the cut of May 1956:

Mn Concentration in Herbage, p.p.m. Dry Matter, Invermay, May 1956

	No Mg	Mg	
Low L	536	380	LSD 5% = 75
High L	173	167	LSD 1% = 99

Magnesium carbonate reduced the very high concentration of manganese appreciably on the low L plots but had no effect on the high L plots. Since it is known that the concentration of manganese in the herbage is greatly influenced by changes in soil pH (although the high L treatment also increased slightly the proportion of *Trifolium repens* in the sward at that time—Table 7), the corresponding soil pH data are given:

Soil pH 0–3 in., February 1956, Invermay

	No Mg	Mg	
Low L	5.8	6.0	LSD 5% = 0.14
High L	7.0	7.0	LSD 1% = 0.18

The Mn concentration in the mixed herbage was reduced by 156 p.p.m. when the soil pH was raised from 5.8 to 6.0 through the use of magnesium carbonate. The high rate of lime increased soil pH to 7.0 and reduced the Mn concentration by a further 213 p.p.m.

The method of pH determination used in this experiment did not measure small pockets of high pH nor the presence of unreacted particles of limestone. It could give, therefore, only an approximate estimate of the soil pH environment in the immediate vicinity of plant roots.

The molybdenum treatment exerted a marked main effect by lowering the Mn concentration in the herbage in three cuts. Molybdenum interacted with the lime treatments as shown at the top of page 973.

The molybdenum treatment reduced the Mn concentration in the mixed herbage on the low L plots but not on the high L plots. The results of the cut of March 1955 could be fairly satisfactorily explained

Mn Concentration in Herbage p.p.m. Dry Matter, Invermay

	March 1955			May 1956		
	Low L	High L		Low L	High L	
No Mo	197	117	LSD 5% = 17	530	174	LSD 5% = 75
Mo	156	107	LSD 1% = 23	387	166	LSD 1% = 99

	April 1958		
	Low L	High L	
No Mo	232	97	LSD 5% = 38
Mo	164	85	LSD 1% = 51

by dilution effects and a change in the proportion of species in the sward (Table 7). Molybdenum markedly increased the proportion of *Trifolium repens* and slightly decreased the proportion of the grasses in the sward.

In the cut of May 1956, however, molybdenum did not increase to any degree the proportion of clovers in the sward nor did it reduce the proportion of grasses. There is therefore evidence that the concentration of Mn in the herbage was affected by the molybdenum treatment on those plots which were deficient in molybdenum but not on the plots which were not molybdenum-deficient. Since the response to molybdenum was not great in autumn 1956 and 1958, a dilution effect cannot be postulated as an important causal factor.

The lowering of the level of manganese in the herbage is probably due to some changes in soil conditions associated with the correction of molybdenum deficiency. A higher nitrogen supply on the Mo low L plots compared with the no Mo low L plots may have been a factor.

Sulphur increased the Mn concentration in the herbage very slightly in two cuts. It is likely that this effect was assisted by the very slight (significant at the 5% level) lowering of pH on plots receiving sulphur.

Marton

The manganese concentration in the herbage was affected by high L, magnesium carbonate, sulphur, high P, and high K. In contrast to Invermay, the molybdenum treatment had no effect. This would be expected if the available soil nitrogen levels were the reason for the effect at Invermay, since at Marton no consistent yield responses to molybdenum were observed.

The high L treatment approximately halved the concentration of Mn, but this main effect, as at Invermay, was complicated by the positive LMg, and negative LK, interactions.

Table 13 summarises these interactions.

TABLE 13. Treatment Interaction Effects on Manganese Concentration of Herbage, Marton

Date of Cut	January 1955	October 1955	January 1956
Type of Interaction	LMg** positive SK * negative BK * positive	LMg** positive LK * negative SK * negative	LMg** positive LK * negative BP ** negative

Date of Cut	November 1956	October 1957 grasses	October 1957 clovers
Type of Interaction	LMg** positive LK * negative SK * negative	LMg** positive LK ** negative BP * negative BK * positive	LMg** positive

* Sig. at 5% level.
** Sig. at 1% level.

By November 1957 the effect of lime and magnesium carbonate treatments on soil pH was as follows:

Soil pH 0–3 in. (Estimated from Values of 0–1½ in. and 1½–3 in. Samples)—Marton, November 1957

	No Mg	Mg
Low L	5.5	5.8
High L	6.3	6.5

The LMg interaction on the Mn concentration in grasses in the cut taken in October 1957 is given below:

Grasses, Mn pp.m. Dry Matter, Marton, October 1957

	No Mg	Mg	
Low L	259	157	LSD 5% = 22
High L	109	87	LSD 1% = 31

There was a much more marked fall in the concentration of Mn in grasses between pH 5.5 and pH 5.8 than between pH 6.3 and pH 6.5.

In the same cut the LMg interaction on Mn concentration in clovers was as follows:

Clovers Mn p.p.m. Dry Matter, Marton, October 1957

	No Mg	Mg	
Low L	117	73	LSD 5% = 13
High L	60	52	LSD 1% = 18

In both grasses and clovers the concentration of manganese was reduced in the same proportion (38–39%) by magnesium carbonate on the low L plots. Heavy liming, however (particularly when added to magnesium carbonate treatment), had a greater relative and absolute effect on the Mn concentration in grasses than in clovers.

In both grasses and clovers there was a marked drop in Mn concentration between pH 5.5 and 5.8. There was a further appreciable decrease between pH 5.8 and 6.3 only in grasses.

The negative LK interaction on the concentration of Mn in the mixed herbage occurred in the cuts of October 1955, January 1956, and November 1956, and in grasses in the cut of October 1957.

The effect is best illustrated in considering the LK interaction on grasses in October 1957:

Grasses, Mn p.p.m. Dry Matter, Marton, October 1957

	Low K	High K	
Low L	188	228	LSD 5% = 22
High L	104	93	LSD 1% = 31

The high K treatment increased the concentration of manganese in grasses in the low lime plots only. The effect of the high K treatment on the Mn concentration may simply be due to the slight lowering of the soil pH in the 0–1½ in. soil horizon (by 0.1 unit, significant at the 5% level). The application of muriate of potash has lowered soil pH slightly in several field trials.

The negative SK interaction on the Mn concentration of the mixed herbage occurred in three cuts. The high K treatment raised the Mn concentration on the no-S plots, but there was little change on the plots receiving sulphur.

Sulphur tended to increase the manganese concentration in the mixed herbage on the low K plots and to decrease the levels on the high K plots. It is possible that this interaction was partly brought about by dilution effects, since there was a strong SK positive interaction on yields.

The Effect of Treatments on the Molybdenum Concentration in the Herbage

Invermay. Chemical analysis of the herbage for molybdenum concentrations was confined to three cuts.

The molybdenum concentration on the low L—no Mo treatments was low: an average of 0.18 Mo p.p.m in herbage dry matter in the cut of December 1953; an average of 0.12 p.p.m. in the cut of May 1956; and an average of 0.25 p.p.m. in April 1958. Many individual plots, however, had levels of only 0.1 p.p.m. in the cuts of December 1953 and April 1958, and of less than 0.05 p.p.m. in the cut of May 1956.

The main effects of treatments are summarised in Table 10. The treatment interactions, without which several main effects cannot be considered, are summarised in Table 14.

TABLE 14. TREATMENT INTERACTION EFFECTS ON MOLYBDENUM CONCENTRATION OF HERBAGE, INVERMAY

Date of Cut	December 1953	May 1956	March 1958
Type of Interaction	LMo** positive KMo** negative LPK** negative	LMo* positive	LMo** positive

* Sig. at 5% level.
** Sig. at 1% level.

Judging by yield results, the high L treatment removed almost entirely any deficiency of available soil molybdenum. It is therefore of interest that its effect on molybdenum concentration in the herbage was relatively slight on the plots not receiving molybdenum.

In the cut of December 1953 the LMo interaction on the molybdenum concentration in the herbage gave the following results:

Mo in Herbage, p.p.m. Dry Matter, Invermay, December 1953

	No Mo	Mo	
Low L	0.18	1.32	LSD 5% = 0.13
High L	0.25	1.69	LSD 1% = 0.18

On the plots without molybdenum, although the high L treatment did not appreciably alter the concentration of Mo, this treatment was effective in removing a clear-cut deficiency of molybdenum.

In the cut of May 1956 the interaction followed a similar course. In the cut of March 1958 the high L treatment had no effect whatsoever on the Mo concentration of the herbage in the plots

without Mo, but it almost doubled the Mo concentration on the Mo plots.

The high P treatment had no direct effect on the concentration of molybdenum, and its role in the LPK negative interaction (Table 14) does not appear to be marked.

The high K treatment reduced the Mo concentration in the mixed herbage in the cut of December 1953, an effect which might have been due to an antagonism between the chloride and the molybdate ion, but could also have been due to a very slight lowering of soil pH. This reduction in the level of Mo concentration was only apparent on the molybdenum-treated plots.

Sulphur decreased the Mo concentration in the herbage in both cuts, which might have been due mainly to an anion antagonism.

Magnesium carbonate markedly increased the Mo concentration in the herbage in the second cut. It is thought that this was mainly the result of a slight reduction in soil acidity.

Marton. Owing to the large amount of work involved, only three cuts of mixed herbage December 1953, January 1955, and January 1956 were analysed for molybdenum. The level of molybdenum concentration in the herbage was much higher than at Invermay, but the treatment effects were very similar.

Both the heavy lime and the molybdenum treatments had marked effects on the Mo uptake of pasture.

The LMo positive interaction was statistically significant at the 1% level in all three cuts. A good example is the interaction table for the January 1955 cut:

Mo Concentration in Herbage, p.p.m. Dry Matter, Marton, January 1955

	No Mo	Mo	
Low L	0.26	2.51	LSD 5% = 0.38
High L	0.56	3.81	LSD 1% = 0.52

Heavy liming doubled the Mo concentration in the herbage on the plots without Mo. This doubling is in contrast with the results at Invermay, where heavy liming had little effect on the Mo concentration on these plots.

Magnesium carbonate increased the Mo herbage concentration quite appreciably in January 1955 and January 1956. There was no LMg interaction; that is, the increase was similar with and without heavy liming, as distinct from the effect of these two pairs of treatments on the Mn concentration.

Double superphosphate, despite its slight molybdenum content (about 6 p.p.m.), had no measurable effect on Mo concentration in the herbage.

The high rate of muriate of potash treatment lowered the Mo concentration in all cuts in which molybdenum was determined. This effect was too large to be explained solely by a dilution effect due to increased growth. As already discussed, the slight lowering of soil pH in the high K treatment, which was recorded in the top 1½in. of soil, may have been the main reason for an increase of manganese uptake in 1956. This could also explain its effect on the reduction in molybdenum concentrations.

The sulphur treatment markedly depressed the Mo concentration in the pasture in the January 1955 and 1956 cuts. On both occasions the SMo negative interaction was statistically significant at the 1% level. In January 1955 results were as follows:

Mo Concentration in Herbage, p.p.m. Dry Matter, Marton, January 1955

	No S	S	
No Mo	0.49	0.32	LSD 5% = 0.38
Mo	3.98	2.34	LSD 1% = 0.52

In the soil samples (0–3 in.) taken in November 1954 sulphur had no appreciable effect on soil pH. Hence it is likely that a specific depressing effect on Mo uptake unconnected with pH was exerted by sulphate ions.

The Effect of Treatments on the Boron Concentration of Pastures. Boron analyses were carried out only once in the cut of December 1953 at Invermay. The concentration of boron was low. On nearly one half of the plots the concentration ranged from 2–10 p.p.m. boron. Sulphur gave a highly significant increase in the boron concentration on the plots receiving borax but not on the plots without borax.

Molybdenum decreased the boron concentration. At the same time molybdenum increased the proportion of *Trifolium repens* and decreased the proportion of *Trifolium pratense* in the sward. It is just possible that this shift in pasture species composition affected the boron concentration of the mixed herbage. In the cut of October 1953 the LMoB interaction on the proportion of *Trifolium repens* was highly significant but the connection between this interaction and the effect of the Mo treatment on the boron concentration of the herbage is obscure.

The 3 tons of limestone per acre applied some 10 months earlier had no effect on the boron concentration in the pasture.

The Effect of Treatment on the Copper Concentration of Pasture. Copper analyses were carried out only once at Invermay on the cut of March 1958.

Heavy liming and the high phosphate treatment depressed the concentration of copper in the herbage. Since, in this cut, only the high P and the high K treatments gave a yield response, it seems likely that heavy liming depressed the availablity of copper in the soil, while the action of high P may have been partly due to a dilution effect.

There were no treatment interactions on the Cu concentrations of herbage.

The Effect of Treatments on Herbage Yields

Main Effects

Since many interactions occurred, the main effects are only of limited interest. For this reason they are not discussed here in detail. One point, however, needs to be made.

The source of phosphorus was TVA double super, which proved to contain 6.5 p.p.m. Mo. Each application of 2 cwt of TVA double super therefore applied 1/17 oz of sodium molybdate equivalent. At Invermay the application of as little as 1/32 oz of sodium molybdate per acre has resulted in marked growth responses on grass-clover pasture lasting for several years (Cullen 1955). It is possible that the fleeting growth response to Mo at Marton at the beginning of the trial (1% sig.) might have continued if a purer source of phosphorus had been used. At Invermay the growth response to sodium molybdate was almost certainly reduced by the use of TVA double superphosphate.

Marton

Table 15 presents a summary of significant main effects of treatments.

Interaction Effects of Nutrients on Herbage Yield. With the very large number of possible interactions in trials of this type there is a high probability that some will attain statistical significance by chance. It is necessary therefore to consider only those interactions which are consistent and those which are highly significant.

Table 16 summarises these interactions. In this and subsequent tables the following abbreviations are used: Sp = spring (September, October, November); Su = summer; A = autumn; W = winter; Y = yearly total.

The LMo and LMoB Interactions on Yield. The LMo negative interaction at Marton is fairly weak, a result which could be expected in view of the absence of a main effect of Mo on yields. The LMoB negative interaction on yields was significant in the second and third years. The interaction table of the total yields for the year 1953–54 is presented at the top of page 981.

TABLE 15. SIGNIFICANT MAIN EFFECTS ON YIELD RESPONSES, MARTON

Year	Mean Trial Yield (lb dry matter/acre)	As Percentages	
1952–53	7,800	Lime 10% **	Phosphate 5% **
1953–54	6,200	Lime 6% ** Potash 9% **	Phosphate 7% ** Sulphur 2%*
1954–55	5,500	Lime 11% ** Potash 9% **	Phosphate 10% ** Sulphur 5% **
1955–56	5,600	Lime 5% ** Potash 8% ** Magnesium 2% *	Phosphate 8% ** Sulphur 4% **
Part year 1956–57	5,250	Lime 6% ** Potash 8% ** Magnesium 4% **	Phosphate 13% ** Sulphur 11% **
Total first four years	25,200	Lime 8% ** Potash 6% **	Phosphate 7% ** Sulphur 3% **

* Significant at 5% level.
** Significant at 1% level.

TABLE 16. SIGNIFICANT INTERACTIONS AFFECTING PASTURE PRODUCTION, MARTON

Type of Interaction	Seasons and Years of Occurrence
LMo negative	A 54*; Sp 54*; W 56*; Sp 56*; Su 56–57*.
LP negative	A 53*; A 54**; Y 53–54**; Sp 54**; W 55**; Y 54–55*; Su 56–57**; Sp 57*; total first 4 yrs**.
LMg negative	Su 52–53*; A 54*; A 55**.
LMoB negative	Y 53–54*; Sp 54*; A 55*; Y 54–55**.
MgP negative	Sp 52–53*; Su 53–54*; A 54*; Y 53–54**; Su 55–56*; total first 4 yrs**; Su 56–57**; Sp 57**; part year 56–57*.
SK positive	Y 53–54*; A 55*; W 55**; Y 54–55**; Sp 55**; A 56**; W 56**; Y 55–56**; total first 4 yrs**; Su 56–57*.

* Significant at 5% level.
** Significant at 1% level.

Herbage Yields (lb dry matter/acre), Marton, 1953–54

	No B		B		
	Low L	High L	Low L	High L	
No Mo	6,133	6,402	5,856	6,563	LSD 5% = 274
Mo	5,968	6,310	6,115	6,301	LSD 1% = 372

There is a negative LMo interaction only on the plots treated with borax.

A similar LMoB interaction occurred in spring 1954 with regard to percentage of rye-grass in the sward.

The LP Interaction on Yield. The interaction table of yield data for the total of the first four years is as follows:

Herbage Yields (lb dry matter/acre), Marton, Total 4 Years

	Low P	High P	
Low L	22,980	25,360	LSD 5% = 635 lb
High L	25,570	26,730	LSD 1% = 860 lb

Yields near maximum were obtained on the high L–low P and on the high P–low L plots. Since heavy liming increased the P concentration in the herbage, it is likely that it exerted a "phosphate sparing" effect. It also increased the level of the Truog soil test for P. This effect has been noted in many field experiments. Since the Truog test is known to extract efficiently phosphorus in the form of calcium phosphates, it is probable that heavy liming led to a shift in the equilibrium of forms of phosphate in the soil in the direction of calcium bound phosphates.

The LK Interaction on Yield. The positive LK interaction at Marton was consistent. There was very little response to high K without adequate lime. The interaction table of yield data for the total of the first four years is as follows:

Herbage Yields (lb dry matter/acre), Marton, Total 4 Years

	Low K	High K	
Low L	23,740	24,600	LSD 5% = 635 lb
High L	25,060	27,250	LSD 1% = 860 lb

The interaction appears to be mainly due to low soil pH limiting the response to potassium and low potassium limiting the response to lime. As heavy liming decreased the potassium concentration in clovers, maximum yields could only be expected in the presence of both high lime and high potassium.

The LMg Interaction on Yield. The negative LMg interaction was not consistent throughout the trial.

The interaction table of yield data for autumn 1955 reads as follows:

Herbage Yields (lb dry matter/acre), Marton, Autumn 1955

	No Mg	Mg	
Low L	1,138	1,255	LSD 5% = 60
High L	1,360	1,359	LSD 1% = 83

Magnesium carbonate caused a highly significant yield response only in the absence of heavy liming.

It is relevant to refer to the effect of the magnesium carbonate and high L treatments on the manganese concentration of herbage and soil pH. In the absence of heavy liming, magnesium carbonate caused a marked drop in the manganese concentration of the herbage; while, in the presence of heavy liming, it had no effect.

Similarly, magnesium carbonate raised soil pH more on the low L plots than on the high L plots.

The LMg interaction, however, cannot be explained entirely by pH effects and resulting depression of toxic Mn concentrations in the herbage. Magnesium carbonate interacted negatively with rates of phosphate with regard to yields (see below). The rates of lime also interacted negatively with rates of phosphate. Therefore this interaction was probably due to two effects—a phosphate sparing effect and a pH effect—acting in the same direction. The LMgP interaction was confounded when designing the experiment, so that a more detailed examination of this three-factor interaction is not possible.

The MgP Interaction on Yield. The interaction table of yield data for the first four years' total reads as follows:

Herbage Yields (lb dry matter/acre), Marton, Total 4 Years

	No Mg	Mg	
Low P	23,790	24,770	LSD 5% = 635
High P	26,180	25,910	LSD 1% = 860

The yield responses to high phosphate were less in the presence of magnesium carbonate than in its absence. There was a highly significant response to magnesium carbonate on the low P plots, but no response on the high P plots. The MgP interaction was somewhat more consistent than the LP interaction.

There is no obvious explanation for the apparent phosphorus sparing effect of magnesium carbonate. It is not likely that magnesium carbonate exerted its effect by a slight shift in soil pH. If this had been the case one would have expected that both the Mg and the high L treatment would have affected the P concentration of the herbage in the same direction. But this did not happen. Heavy liming consistently increased P concentration; magnesium carbonate did not. In the cut of December 1953 it lowered the P concentration in the herbage to a highly significant degree.

An improvement by magnesium fertilisers in the efficiency of phosphorus utilisation by the plant would not be expected on a soil such as the Marton loam, which appears, from plant analysis levels, to be already well supplied with available magnesium.

The SK Interaction on Yield. The positive SK interaction occurred consistently after the first year of the trial. The interaction table of yield data for the four-yearly total reads as follows:

Herbage Yields (lb dry matter/acre), Marton, Total 4 Years

	No S	S	
Low K	24,400	24,400	LSD 5% = 635
High K	25,230	26,620	LSD 1% = 860

A yield increase from sulphur was obtained only on the high K plots. The growth response to high K was much more marked on the sulphur plots. The interaction appears to be of the limiting factor type. It is, nevertheless, surprising that there is so little evidence of SK interaction with regard to the proportion of either white clover or perennial rye-grass in the sward. The high K treatment apparently increased the proportion of white clover equally well with and without sulphur.

The addition of sulphur would seem necessary, however, to increase the rate of growth of the potash-treated plots.

The LSK Interaction on Yield. The LSK interaction was not confounded by the experimental design.

The yield responses to high K at Marton are shown at the top of page 984.

The effect of S and rates of lime on the yield response to high K is additive. The LSK interaction, therefore, cannot be significant.

Total 4 Years

	Percentage Response to High K
On low L, no S plots	0
On low L, S plots	8
On high L, no S plots	7
On high L, S plots	15

The LPK Interaction on Yield. The LK interaction was positive and the LP interaction negative. Lime responses, therefore, varied according to the combination of rates of K and P applied.

The responses to high L at Marton were as follows:

Total 4 Years

	Percentage response to High L
High P, low K	3 (not significant)
High P, high K	7
Low P, low K	8
Low P, high K	15

The effect of rates of P and K on the yield response to high L is additive, so that the three-factor interaction could not be expected to be significant.

Invermay

In contrast to the Marton trial, sulphur and high K treatments had very little effect on yields, while molybdenum increased yields consistently.

In Table 17 the main effects of treatments are summarised.

TABLE 17. SIGNIFICANT MAIN EFFECTS ON YIELD RESPONSES, INVERMAY

Year	Mean Trial Yield (lb dry matter/ acre)	Responses as Percentages							
1953/54	6,000	Mo	8%**	B	— 4%*	Lime	4%*	P	13%**
1954/55	7,600	Mo	9%**			Lime	8%**	P	9%**
1955/56	7,000	Mo	4%**			Lime	12%**	P	6%**
1956/57	8,900	Mo	6%**	Mg	3%*	Lime	13%**	P	7%**
1957/58	9,800	Mo	3%*			Lime	4%*	P	10%**
1958/59	7,800	Mo	12%**	Mg	7%**	Lime	20%**	P	11%**
Mean 1953/59	7,900	Mo	7%**	Mg	3%*	Lime	10%**	P	10%**

* Significant at 5% level.
** Significant at 1% level.

As at Marton, most treatments participated in interactions, so that a discussion of main effect only would be of little interest. The consistent treatments of interactions on pasture yield are summarised in Table 18.

TABLE 18. Significant Interactions Affecting Pasture Production, Invermay

Type of Interaction	Seasons and Years of Occurrence
LMo negative	Su 54–55*; Y 54–55*; Su 55–56*; Su 56–57*; Y 56–57*; Sp 57–58**; Y 57–58*; W 58**; Sp 59**; Su 58–59**; Y 58–59*; 6 years 53–59**.
LP positive and later negative	Su 53–54 (+)**; A 54 (+)**; W 57 (—)**; A 58 (—)*; Y 57–58 (—)*; W 58 (—)*.
LMg negative	W 56*; Su 56–57*; Y 56–57*; Su 57–58*; A 58*; W 58*; Sp 58**; Su 58–59**; Y 58–59**; 6 years 53–59*.
LS positive	Sp 53*; A 54**; Y 53–54*; Sp 57*; Su 58–59**.
LPS positive	Sp 53**; Y 53–54*; Sp 54*; A 55*; Y 54–55*.
MgMo negative	W 58*; Sp 58*; Su 58–59*; Y 58–59*.
LMgMo positive	Y 58–59**.
BP positive	Su 56–57*; Y 56–57*; Sp 57*; Sp 58*.
LBP negative	Sp 53*; Sp 57*; W 58*.

* Significant at the 5% level.
** Significant at the 1% level.

The LMo Interaction on Yield. As would be expected on a molybdenum-deficient site, the negative LMo interaction is more consistent at Invermay than at Marton. In the absence of molybdenum high L gave a good response, whereas there was a much smaller response to high L in the presence of molybdenum. Molybdenum gave a good yield response on the low L plots; but it gave little, and, at times, no response in the presence of high L. The following results were obtained for the six-year period 1953–59:

Herbage Yields (lb dry matter/acre), Invermay, Total 6 Years

	Low L	High L	
No Mo	41,870	48,250	LSD 5% = 2,200
Mo	46,960	49,500	LSD 1% = 3,000

There is little doubt that the separate effects of molybdenum and lime were due to increased nitrogen fixation by clovers and to increased clover growth, as supported by the negative LMo interactions recorded on the proportion of *Trifolium repens* in the sward. As mentioned previously, it is likely that the yield response to high L in the presence of molybdenum may have been partly due to reducing the manganese concentration, particularly of the grasses. The assessment of the factors leading to the lime response is further complicated by the negative LP interaction occurring in the years 1957 and 1958. This interaction would indicate that in those years the high L treatment derived much of its effect on yield from the greater availability of phosphorus on the low P plots.

The LP Interactions on Yield. These interactions are of particular interest, since they were positive in the first year and negative later on. The positive interaction was probably of the limiting factor type. The plots receiving both the high rate of lime and the high rate of phosphate were by far the best. There was little response to high L on the low P plots or to high P on the low L plots.

The negative LP interaction is well illustrated by the yield results for the year 1957–58. By this time the Truog test P level on the low P plots was higher than the test on the high P plots in 1954 (in the second year of the trial).

Herbage Yields (lb dry matter/acre), Invermay, 1957–58

	Low L	High L	
Low P	8,709	9,344	LSD 5% = 374
High P	9,882	9,956	LSD 1% = 510

The high L treatment did not produce a yield response on the high P plots and the yield response to high P was much reduced on the high L plots.

The LMg Interaction on Yield. The LMg interaction did not appear until the third year, but from then onwards it was consistent. Magnesium carbonate gave a response on the low L plots but not on the high L plots.

The LS and LPS Interactions on Yield. The positive LS interaction occurred fairly consistently.

Sulphur depressed yields on the low L plots but increased yields on the high L plots. Sulphur also increased the proportion of *Trifolium repens* on the high L plots on one occasion. It appears, therefore, that the site was slightly deficient in available sulphur. On the low L plots, however, the slight advantage of sulphur in correcting a marginal sulphur deficiency appeared to be more than offset by its adverse effect on molybdenum availability.

The LPS interaction on yields which occurred in the first two years expressed itself as follows. On the high P plots there was a positive LS interaction, while on the low P plots there was no LS interaction. This is consistent with deficiencies of all three elements, especially P.

The MgMo and LMgMo Interactions on Yields. In the final year negative MgMo interactions occurred consistently. There was a good yield response to magnesium carbonate on the plots without Mo, but only a very slight response on the Mo-treated plots. The interaction was probably due to a pH effect.

The changes in pH due to the lime and magnesium carbonate treatments in the soil samples (0–3 in.) taken in March 1959 are summarised below.

Soil pH (0–3 in.) Invermay, March 1959

	Low L	High L	
No Mg	5.9	7.0	LSD 5% = 0.13
Mg	6.2	7.1	LSD 1% = 0.17

The change in pH probably released enough available molybdenum to result in a negative MgMo interaction on yields.

In order to assess the possible influence of pH on factors other than available molybdenum, the LMgMo interaction was examined for the year 1958–59; it proved highly significant. The following table summarises the results.

Herbage Yields (lb dry matter/acre), Invermay, 1958–59

	No Mo		Mo		
	Low L	High L	Low L	High L	
No Mg	5,370	8,370	7,610	8,570	LSD 5% = 596
Mg	7,210	8,320	7,890	8,620	LSD 1% = 808

There was a very appreciable response to magnesium carbonate on the low L–no Mo plots, but little, if any, response to magnesium carbonate on the low L–plus Mo plots. There was no response to magnesium carbonate on the high L plots, whether or not they had received Mo. It appears, therefore, that the main value of the magnesium carbonate treatment consisted in releasing available molybdenum. At the same time, it is evident that the much more marked change in pH due to heavy liming had an appreciable effect on yields, even on the plots receiving molybdenum.

The BP Positive and LBP Negative Interactions on Yield. The BP positive interaction occurred occasionally from summer 1956-57 to spring 1958. It was significant at the 5% level for the year 1956-57.

The results of this interaction for the year 1956-57 are given below:

Herbage Yields (lb dry matter/acre), Invermay, 1956–57

	Low P	High P	
No B	8,690	8,967	LSD 5% = 354
B	8,436	9,382	LSD 1% = 481

Borax increased the yield on the high P plots, but not on the low P plots.

It is likely that the site at Invermay was somewhat low in available soil boron. This is also indicated by the low mean concentration of boron in the herbage (11 p.p.m.) in the cut of December 1953.

The LPB interaction, however, gave a strange result; this is best illustrated by the interaction table below, which presents the yields for late autumn-winter 1958:

Herbage Yields (lb dry matter/acre), Invermay, Winter 1958

	Low L		High L		
	Low P	High P	Low P	High P	
No B	963	1,366	1,310	1,740	LSD 5% = 220
B	803	1,670	1,331	1,614	LSD 1% = 300

Borax gave a highly significant rise in yields on the high P–low L plots, but not on the high P–high L plots. This is contrary to expectations, since a high soil pH is usually regarded as conducive to boron deficiency. However, the possibility that the limestone used contained traces of boron cannot be discounted. Recently a limestone obtained by the Department of Agriculture for experimental purposes was found to contain about 100 p.p.m. of boron.

The Invermay trial did not furnish evidence that boron is required for pasture growth on well-limed and well-phosphated soils, but it indicated: (a) that a marginal boron deficiency on pasture might occur on this and related soil types; and (b) that, in field trials with boron, care should be taken to supply phosphatic top-dressing at an adequate rate of application.

A positive BP interaction also occurred three times at Marton. This suggests that a relatively high level of available soil phosphorus

may be essential on a wide range of soils before marginal boron deficiencies can be discovered.

Seasonal Effects on Treatments of Pasture Yields

Marton

The seasonal effects of treatments on pasture yields at Marton are summarised in Table 19. Fertilisers were applied in November of each year.

TABLE 19. Mean Seasonal Responses to Treatments: Main Effects, 1952–56, Marton

Season	Approx. Mean Yield	Mean Responses to Nutrients (lb dry matter/acre)			
		High P	High L	High K	S
Summer	1,800	160	130	170	—*
Autumn	1,400	170	200	25	70
Winter	500	70	90	40	20
Spring	2,400	100	70	170	30

* Summer responses too variable. A figure for mean response would be misleading.

Phosphate. The high P plots gave the largest responses in absolute terms in summer and autumn, and the largest relative responses in autumn and winter. Spring responses were erratic and were significant only in two years out of four. In the two years in which yield increases were significant in spring the positive BP interaction occurred. It is perhaps no coincidence that a similar BP interaction on the proportion of rye-grass occurred in a cut taken in spring 1955.

The negative MgP interaction was significant in three summers and one autumn.

There was a general trend, during the duration of the trial, for phosphate responses to improve in autumn and winter.

Lime. The yield responses to the high L treatment were similar in distribution to those of phosphate. Significant responses occurred only twice in spring and in the same years as the responses to high P. The summer responses were erratic, but there was a very good response (22%) in the dry summer of 1954-55. High L produced more consistently good winter responses than any other treatment. This effect was very likely due to the higher proportion of Poa species on the high L plots.

Sulphur and Potassium. Sulphur and potassium responses were affected by the consistent and marked SK interaction. In the presence of high K the average responses to sulphur were about 100% higher than those given in the Table, and, in the presence of high S, the average response to high K was about 50% higher. Nevertheless, the

most consistent responses to sulphur were obtained in autumn, while the best and most consistent responses to high potash were obtained in spring and summer, the autumn responses to high K being relatively small. The high K treatment, on the plots with sulphur, stimulated growth in spring and summer much more than any other treatment. Since all treatments were applied in November (the last month of spring), the time of top-dressing with potash would, if anything, minimise the growth-stimulating action of potash during the spring months.

Invermay

Fertilisers were applied in autumn of each year. The very low winter production at Invermay had to be included with autumn production.

TABLE 20. Mean Seasonal Responses to Treatments: Main Effects 1954–59, Invermay

Season	Approx. Mean Yield	Mean Responses To Nutrients (lb dry matter/acre)		
		High P	High L	Mo
Summer	2,700	180	290	180
Autumn and Winter	1,200	300	250	170
Spring	3,700	220	290	270

The seasonal effect on the high P treatment was similar to that at Marton: the greatest response in absolute and relative terms occurred in the autumn-plus-winter period.

The seasonal effect of the high L treatment was fairly evenly distributed over the three periods. In this it differed from Marton where the responses in spring and summer were smaller than at Invermay.

The molybdenum treatment also caused good growth responses in spring. Perhaps, therefore, the difference between the two localities in the seasonal effect of heavy liming is partly due to differences in the level of available molybdenum in the soil. At Invermay the negative LMo interaction on yields occurred six times in spring or summer but only once in the autumn and winter period. All treatments gave the highest relative yield responses in the autumn-plus-winter period.

Discussion and Conclusions

Complicated trials of the type described are very suitable for pilot studies. Their greatest advantage is the precision with which they can measure main effects and two-factor interactions. To do this two conditions need to be satisfied:

(a) Deficiencies must not be severe enough to place a serious limit on the magnitude with which other treatments can affect growth and species composition. Severe deficiencies were successfully avoided in these trials.

(b) Deficiencies must not be too many, so that two-factor interactions are not weakened appreciably by high order interactions which are confounded in the design of the trial.

It is probable that, in these trials, some of the two-factor interactions were weakened by high order interactions.

Keeping in mind these limitations, which are unavoidable if a large number of treatments is to be examined factorially in a small space, the following conclusions can be drawn.

Inter-relationships between yields, botanical composition of pasture, concentration of nutrients in the herbage, and soil test results were measured with considerable accuracy. This has given a closer insight into the nature of fertiliser and lime responses on these soils.

Heavy liming, 3 tons per acre, was compared with very light liming at 3 cwt per acre. Heavy liming raised soil pH and the Truog P value and depressed the value of the soil K quick test. It increased the concentration of calcium, molybdenum, and phosphorus in the herbage, and decreased the concentration of potassium, magnesium, and copper (Invermay). At Invermay, on a molybdenum-deficient soil, it increased the proportion of *Trifolium repens* and *Lolium perenne* in the sward, while at Marton it increased the proportion of Poa spp. It increased yields at Invermay mainly because it raised the availability of soil molybdenum and, to a lesser degree, that of soil phosphorus. At Marton heavy liming exerted a phosphate sparing effect but it also assisted to increase responses to high K and to sulphur. Thus the yield responses to high L were primarily due to an increase in the availability of molybdenum and phosphorus and to a better utilisation of elemental sulphur and potassium chloride. It is not known how much heavy liming contributed to increased yields by reducing available manganese.

Magnesium carbonate, applied annually at the rate of 3 cwt per acre, raised the soil pH very slightly. It increased the concentration of magnesium and molybdenum in the herbage and decreased the concentration of potassium, calcium, and manganese. It is considered that, after several annual applications at Invermay, it corrected molybdenum deficiency to a considerable degree, with the result that the proportion of *Trifolium repens* in the sward was increased and yields were slightly improved. At Marton it also increased yields, but for a different reason—it had an apparent phosphate sparing effect. There was no evidence of a magnesium deficiency on either site.

Muriate of potash at 2 cwt per acre each year was compared with $\frac{1}{2}$ cwt per acre per annum. The heavy rate increased the K soil test, especially at Invermay. At Marton it increased the proportion of *Trifolium repens*.

It increased the concentration of potassium in the herbage and decreased the concentration of phosphorus, sodium, magnesium, calcium, and molybdenum.

It increased yields at Marton, particularly on plots which were both supplied with sulphur and heavily limed; but it did not increase yields on plots receiving neither sulphur nor a heavy rate of lime. Growth responses were most marked in spring and summer.

Double superphosphate (changed later to Ibex brand monocalcium phosphate at Invermay), at 2 cwt per acre per annum, was compared with $\frac{1}{2}$ cwt. The heavy rate increased the Truog P soil test value. It had no effect on pasture composition at Marton but increased the proportion of *Lolium perenne* and *Trifolium repens* in sward at Invermay. It increased the concentration of phosphorus and sodium in the herbage and decreased the concentration of potassium.

It increased yields, particularly on the low L plots. Growth responses were most marked in autumn and winter.

Sodium molybdate at $2\frac{1}{2}$ oz per acre had little effect at Marton. At Invermay it increased the proportion of *Trifolium repens* and decreased the proportion of *Trifolium pratense*. It also increased the concentration of molybdenum and nitrogen in the herbage, but decreased the concentration of manganese. At Invermay sodium molybdate gave a clear yield response on the low L plots, but it gave little, and, at times, no, response on the high L plots. Yield responses were largest in spring.

Sulphur, even at the rate of 56 lb per acre per annum at Invermay, did not affect soil pH appreciably, yet it had a pronounced depressing effect on the molybdenum uptake by plants. It also decreased the concentration of phosphorus and potassium in the herbage at Marton; increased the concentration of manganese on both sites; and increased the concentration of boron at Invermay.

Quite a good yield response to sulphur was obtained at Marton, particularly on the high L–high K plots. On the low K plots sulphur yield responses were slight or absent.

Borax increased the concentration of boron in the herbage at Invermay. At both sites there was some evidence that borax increased yields occasionally on the high P plots, but not on the low P plots.

The results from these trials point to new lines of investigation in the field of plant nutrition; they also indicate the importance of balancing fertiliser and lime applications correctly for maximum returns.

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INSECTS ASSOCIATED WITH THE MAJOR FODDER CROPS IN THE NORTH ISLAND

II. HEMIPTERA

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Summary

A survey of fodder crops in the North Island, carried out during 1959, revealed 50 species of Hemiptera. Two are new records for New Zealand. The identity, distribution, incidence, and possible economic importance of each species are discussed. A list of the species occurring on each crop is appended.

Introduction

A survey of the insects associated with fodder crops in the North Island was carried out during the summer of 1959. A general account of this survey has already been presented (Eyles 1960). The major fodder crops sampled were lucerne, maize, chou moellier, swedes, mixed chou moellier and swedes, and turnips. There were, in addition, minor samplings of mangels, rape, pampas, and mixtures of crucifers.

The present paper lists the species of Hemiptera taken in the survey, and gives information on their distribution, incidence on the various crops, habits (where not covered in Cumber's pasture survey 1959), and economic significance.**

The majority of species were represented by few specimens. Distribution maps for the seven species most commonly encountered are given, a black square indicating at least one occurrence within the area.

Results

Hemiptera-Homoptera

Fam. Cicadidae

The cicada larval stages are spent below the surface of the soil, where nymphs feed upon roots of various plants. Here they may be regarded as damaging, but they may be more obviously damaging as adults when they affect aerial portions by feeding, and, more especially, with their oviposition scars.

He. 58. *Melampsalta muta* (Fabr.)

This indigenous species is widely distributed in both North and South Islands, and is present in the Chatham Islands (Myers 1929). In the present survey two adults were taken from pampas at Kotemaori on 9.2.59. Myers states that *M. muta* occupies a mesophytic but open

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** Where further information is contained in Cumber's pasture account it is indicated by an asterisk after the species.

country habitat. The species is of no economic importance on fodder crops.

He. 57. *Melampsalta sericea* (Walk.)

Myers (1929) shows that this endemic species is restricted to the North Island. In the present survey, a single adult was collected from maize at Dargaville on 26.2.59. Myers records the ecological habitat of this species as dry open spaces and exposed banks. *M. sericea* is of no economic importance on fodder crops.

Fam. JASSIDAE (Leaf-Hoppers)

This is a very extensive family of economically important insect species. Cereal and fodder crops, and fruit and forest trees, are commonly affected; injury results from direct mechanical means, production of toxins, or spread of virus diseases.

He. 5. *Erythroneura zealandica* Myers*

This species is widely distributed in New Zealand. The distribution shown by the present survey is given in Fig. 1, which indicates

Fig. 1

Fig. 2

Fig. 1.—Distribution map for the jassid *Erythroneura zealandica* He. 5.

Fig. 2.—Distribution map for the aphid *Brevicoryne brassicae* He. 8.

a mainly southern distribution in the North Island. It was taken on lucerne (5 crops), maize (6), chou (4), chou plus swedes (1), turnips (1), swedes (1), and rape (1). It was never taken in large numbers, however—seven on lucerne (Greytown 8.4.59) and five on maize

(Shannon 25.3.59) being the greatest catches. This situation contrasts strongly with that met in pastures, where very considerable numbers were taken. This species is not considered to be important in fodder crops, except perhaps in possible virus transmission in lucerne and maize.

He. 19. *Deltocephalus* sp.*

Only one specimen (maize, Taradale 9.2.59) of this widely-distributed species, which is commonly present in pastures, was taken.

He. 13. *Thamnotettix* sp.*

This species is widely distributed south of Auckland in the North Island. In the present survey a single specimen was taken from maize at Taradale on 9.2.59. Damaging members of this genus occur on cereals in North America (Osborn 1939). This species occurred commonly in pasture sweeping.

He. 14. *Thamnotettix* sp.*

A single specimen of this species, which is widely distributed in the North Island, was collected from turnips at Makerua on 25.3.59.

He. 11. *Xestocephalus* sp.*

The genus is cosmopolitan. Cumber (1959) shows that the distribution of this species in pastures is south of the Bay of Plenty. Two specimens were collected in the present survey—one from lucerne at Bulls and one from maize at Pio Pio (both on 16.3.59).

He. 54.? *Idiocerus* sp.

The genus *Idiocerus* occurs in Australia and New Zealand (Tillyard 1926). In the present survey an adult and nymphal exuvium were collected from mangels at Ashhurst on 26.3.59. This was probably associated with Lombardy poplars at one end of the field rather than with the crop.

He. 55. Undetermined.**

One adult and a nymph of this as yet undetermined species were collected from lucerne at Greenmeadows on 9.2.59. The habits of the species are not known.

Fam. Delphacidae

The family includes many species which are injurious to agricultural crops.

He. 7. *Delphacodes tasmani* Muir*

This species occurs throughout the North Island. In the present survey one adult was taken from lucerne at Greenmeadows (9.3.59), and a nymph from maize at Tauranga on 4.3.59.

He. 4. *Delphacodes* sp.*

This species occurs in the North Island on pastures from widely-separated localities. A single adult was taken from turnips at Te Puke on 4.2.59.

** Undetermined species are held under the numbers shown, in the collections of the Entomology Division sub-station, Palmerston North.

Fam. DICTYOPHARIDAE

The family is of minor economic importance.

He. 21. *Thanatodictya tillyardi* Myers*

Cumber (1959) records this species in pastures from four widely separated North Island areas. In the present survey small numbers were taken from turnips at Te Teko on 12.2.59, and from maize at Whangarei on 26.2.59. Myers (1923) and Cumber both record bracken fern (*Pteridium esculentum* Hook) as host plant. The turnip crop referred to above was bounded on one side by manuka (*Leptospermum scoparium* Forst.); while the maize crop, on a small hillside area, was surrounded by rough grasses and weeds which included blackberry (*Rubus* sp.), dock (*Rumex* sp.), inkweed (*Phytolacca octandra* L.), pennyroyal (*Mentha peledeum* L.), and thistles. The species was probably associated with these attendant weeds.

Fam. FLATIDAE

The family is of minor economic importance.

He. 35. *Sephena cinerea* Kirk*

This introduced Australian species is widely distributed in the North Island and the north of the South Island. A small number of adults were taken from lucerne at Bulls (16.3.59) and Ohingaiti (20.3.59), maize at Mangamuka (23.2.59), chou at Cheltenham (24.3.59), and pampas at Kotemaori (9.2.59). The species is known to attack a wide variety of plants in New Zealand, but no detailed studies have been made. It is unlikely to prove of importance in fodder crops.

Fam. RICANIIDAE

The family is of minor economic importance.

He. 28. *Scolypopa australis* (Passion-vine Hopper)*

This Australian species is widespread in the North Island and in the north of the South Island. Single adults were collected from maize at Kaitaia and Mangamuka (23.2.59), from turnips at Te Teko (12.2.59), and from chou plus swedes at Waihi (3.3.59) and Mahoenui (16.3.59). This occasionally associated species is not important in fodder crops. The heavy populations encountered on native vegetation must cause considerable harm, however.

Fam. PSYLLIDAE

The family includes some important pests of agricultural crops.

He. 31 ? *Ctenarytaina pollicaris* Ferris and Klyver*

This species occurs in North and South Islands. A single adult was taken from lucerne at Te Awamutu on 19.3.59. There was no *Leptospermum*, which is regarded as the usual host plant (Tuthill 1952), in the vicinity.

He. 59. *Trioza* sp.

A single adult was taken from mixed chou and swedes at Te Raumoa on 19.3.59.

He. 63. *Psylla* sp.

Three adults were collected from chou at Whakatane on 12.2.59. These were probably associated with the gross weeds rather than with the crop.

Fam. Aphididae

This is an extensive family. Although there are only a limited number of endemic species, many overseas introductions are present in New Zealand.

He. 42. *Aulacorthum solani* (Kltb.)*

This species was taken from a mixed chou and swede crop at Konini on 8.4.59. It has a great number of host plants, and its occurrence is widespread in both Islands.

He. 8. *Brevicoryne brassicae* (L.)*

This world-wide species, commonly a series pest of cruciferous crops, is of widespread occurrence in both Islands. It occurred in 35 cruciferous crops (Fig. 2), being taken from chou, rape, swedes, and turnips, during February, March and April.

He. 41. *Capitophorus elaeagni* (Del Guerc.)*

This world-wide species is known to occur in both Islands, on a number of host plants. In the present survey it was taken from lucerne at Takapau 9.2.59, Otorohanga 19.3.59, and Longburn 24.3.59; from chou mixed with swedes at Pio Pio 16.3.59 and Rauamoa 19.3.59; and from turnips at Masterton 8.4.59.

He. 6. *Hyperomyzus lactucae* (L.)*

This world-wide species is apparently of widespread occurrence in the North Island. The present survey yielded specimens from maize at Dargaville 26.2.59 and Whangarei 26.2.59.

He. 46. *Lipaphis erysimi* (Kltb.)*

This species, which is of world-wide occurrence, was taken from chou at Cambridge 3.3.59 and Urenui 16.3.59; from chou mixed with swedes at Wharepaina 4.3.59; and from turnips at Ohingaiti 20.3.59. It is widely distributed in the North Island, and is known on occasion to be damaging to cruciferous plants.

He. 44. *Macrosiphum avenae* (F.)*

This world-wide species is widely distributed in the North Island. It was taken from turnip crops at Puketona 24.2.59 and Silverdale 2.3.59.

He. 45. *Macrosiphum euphorbiae* (Thos.)*

This world-wide species has been reported from many host plants in both Islands. It was taken from maize at Pakaraka 24.2.59.

He. 47. *Macrosiphum sonchi* (L.)*

This world-wide species is found on thistles. It was taken probably from the associated weeds, in crops of chou at Ashurst 26.3.59; chou mixed with swedes at Maungaturoto 26.2.59 and Konini 8.4.59; maize at Waipawa 9.2.59; swedes at Makerua 26.3.59 and Pahiatua 8.4.59; and turnips at Marton 24.3.59, Te Arakura 24.3.59, and Tokomaru 25.3.59.

He. 65. *Myzus ornatus* Laing

This species was taken from lucerne at Te Awamutu on 19.3.59. Cottier (1953) records it from *Trifolium subterraneum* L., *Lavatera arborea* L., *Primula* sp., *Anemone* sp. and *Bellis perennis L.*, at Palmerston North.

He. 37. *Myzus persicae* (Sulz.)*

This is a world-wide species that is widely distributed in New Zealand. In the present survey it was taken on 35 occasions during February, March, and April, mainly from chou, rape, swedes, and turnips (Fig. 3). It is known to have a great number of host plants and may often reach damaging proportions.

Fig. 3

Fig. 4

Fig. 3.—Distribution map for the aphid *Myzus persicae* He. 37.

Fig. 4.—Distribution map for the aphid *Rhopalosiphum padi* He. 39.

He. 64. *Rhopalosiphum maidis* (Fitch)

This species was taken from maize at Kaitaia 23.2.59, Whirinaki 25.2.59, and Ngawha 25.2.59, and from chou at Pakaraka 24.2.59. Cottier (1953) records it from maize at Auckland and Palmerston North, and from *Avena sativa* at Palmerston North. He remarks upon its occasional occurrence and non-economic importance.

He. 39. *Rhopalosiphum padi* (L.)*

This world-wide species is of widespread occurrence in New Zealand. At times it is troublesome in grain crops (Cumber and Todd 1959). In the present survey it was taken on 18 occasions (Fig. 4), during February, March and April. It occurred for the most part on maize crops.

He. 38. *Rhopalosiphum splendens* (Theo.)*

This world-wide species is of widespread occurrence in the North Island. In the present survey it was taken from chou at Martinborough-Greytown 8.4.59, and from turnips mixed with chou at Greytown 8.4.59.

Hemiptera-Heteroptera

Fam. Nabididae

The majority of species in this family are predacious, many being regarded as highly beneficial insects.

He. 12. *Nabis capsiformis* Germar*

This species, now nearly cosmopolitan, is widely distributed in New Zealand. In the present survey it was taken in most North Island districts (Fig. 5).

A total of 56 adults and 29 nymphs was collected from lucerne (10 crops), maize (7), chou (7), mixed chou and swedes (1), turnips (4), and swedes (2). Numbers in excess of 3 were taken in 5 lucerne crops, in one swede crop. and one turnip crop. Higher numbers included 9 adults on lucerne at Gisborne (10.2.59), and 8 adults on swedes at Pirinoa (8.4.59).

Adults and nymphs were present from early February to early April. This species is actively predacious on other insects and must be regarded as beneficial.

He. 16. *Nabis maoricus* F. Walker*

This endemic species occurs throughout New Zealand. In the present survey it was taken from lucerne at Bulls (3 adults 16.3.59) and Longburn (2 adults 24.3.59). In testing sampling techniques, single adults were taken at Palmerston North from turnips and mature chou late in January 1959. This species must be regarded as beneficial.

Fam. Miridae

This very large family of insects contains many pests that damage agricultural crops. A few beneficial predacious species are known.

Fig. 5

Fig. 6

Fig. 5.—Distribution map for the nabidid *Nabis capsiformis* He. 12.

Fig. 6.— Distribution map for the mirid *Eurystylus australis* He. 17.

He. 27. *Calocoris norvegicus* (Gmelin)*

This European species is now well distributed in the North Island. A single specimen was taken from chou at Cheltenham on 24.3.59. It is probable that this species, if shown to be damaging, will be important in the early part of the season.

He. 26. *Deraeocoris maoricus* Woodward*

This species is widely distributed in the North Island, and occurs in the north of the South Island. In the present survey it was taken in small numbers from turnips at Marumaru (10.2.59) and Rangiriri (3.3.59), and from maize at Shannon (25.3.59). This species, which was taken in greater numbers in pastures, is not considered to be important in fodder crops.

He. 17. *Eurystylus australis* Poppius*

This Australasian species occurs throughout the North Island, and in the north of the South Island. In the present survey it was taken in all North Island districts (Fig. 6).

A total of 82 adults and 36 nymphs was taken from 67 crops, the species being present on lucerne (10 crops), maize (16), chou (12), chou plus swedes (7), turnips (12), swedes (3), mangels (4), rape (1), and mixed rape, swedes and turnips (2). The insect occurred

in excess of 3 individuals in 17 crops, of which 7 were turnips (apparently a major host). The greatest catch, however, was from chou at Mercer on 3.3.59 (18 adults and 6 nymphs).

Adults were taken for the duration of the survey, i.e., from early February to early April. The species occurred far more frequently on fodder crops than in pastures, being the most common hemipteron taken. Although crop damage by this insect was not evident, the species may prove to be more important than is at present realised: its feeding habits make it a potential transmitter of virus or fungus disease, or a potential pest through causing mechanical injury.

He. 18. *Halticus tibialis* Reuter*

This widespread Javanese species is well distributed in northern North Island districts. In the present survey small numbers were taken from lucerne at Te Awamutu (19.3.59); maize at Kamo (2.3.59), Eureka (3.3.59), and Paparoa (4.3.59); and turnips at Te Teko (12.2.59) and Waitoa (3.3.59). This species, which occurred in far greater numbers in pastures, does not appear to be of importance in fodder crops.

He. 50. *Lygus* sp.

Two specimens taken from maize at Hangaroa on 10.2.59 have been determined by Dr T. E. Woodward as *Lygus* sp. He indicates (pers. comm.) that it is impossible to give specific names to New Zealand *Lygus* until the genus in this part of the world has been revised. He believes that New Zealand probably has several undescribed species.

He. 10. *Megaloceroea reuteriana* White*

This species is widespread in Australia and New Zealand. Cumber (1959) collected it in moderate numbers from pastures and showed that it is well distributed in the North Island on such herbage. In the present survey it was taken only from maize—one adult and one nymph at Awarua on 26.2.59, and one adult at Helensville on 2.3.59.

Fam. Anthocoridae

The members of the family are predacious, for the most part upon small arthropods.

He. 51. *Lyctocoris campestris* Fabr.

This predacious species is now almost cosmopolitan (Dr T. E. Woodward, pers. comm.). In the present survey a single adult was taken from maize at Taradale on 9.2.59. If beneficial, this species plays a very minor role.

He. 52. *Poronotellus whitei* Reuter

This species is widespread in Australia and at least the North Island of New Zealand (Dr T. E. Woodward, pers. comm.). In the present survey single adults were taken from maize at Taradale (9.2.59) and Ohinewai (3.3.59). The species is unimportant in fodder crops, but, being predacious, could play a minor beneficial role.

Fam. LYGAEIDAE

The family includes many pests that damage agricultural crops.

He. 9. *Nysius huttoni* White*

This endemic species is widely distributed in the North and South Islands. Single adults were collected from lucerne at Waipawa (9.2.59), Te Awamutu (19.3.59), and Martinborough (8.4.59), and a nymph was taken from chou at Cambridge (3.3.59).

This species, which lives close to the ground surface, prefers areas where vegetation is sparse and where the sunlight strikes through to the ground. Myers (1921) records it on lucerne, and Gurr (1952, 1957) records it as damaging crucifer seedlings in the South Island. It appears that the population does not build up to pest proportions in the North Island. The dry and sparse "edge" vegetation which favours the insect may be a more common feature of the South Island crops. Detailed information on the biology and ecology of this insect is given by Eyles (1958).

He. 23. *Pachybrachius nigriceps* (Dallas)*

This species is widespread in the Pacific area, and is well distributed in the North Island. In the present survey it was taken from maize at Dargaville (1 adult 26.2.59) and Papamoa (2 adults 4.3.59). It is unlikely to prove of importance on fodder crops.

He. 34? *Rhypodes clavicornis* (F.)

The genus is endemic. In the present survey a single adult was taken from lucerne at Greytown on 8.4.59. Although this species is commonly found on some garden plants there is no evidence that it is important in crops.

Fam. PENTATOMIDAE

The family includes many important agricultural pests and some predacious species which must be regarded as beneficial.

He. 61. *Antestia orbona* Kirkaldy

This species is an introduction from Australia. Woodward (1953) recorded material collected near Hastings in March 1950. In the present survey a specimen was taken from the vicinity of a turnip crop at Masterton on 8.4.59. Other adults have recently been collected from bush at Palmerston North (27.9.58, J. M. Esson, and 30.4.59, A.C.E.). These records indicate a wide distribution, at least in the southern part of the North Island. The species is unlikely to prove of importance in fodder crops.

He. 1. *Dictyotus caenosus* (Westwood)*

This species occurs in Australia, and throughout the North Island and northern portion of the South Island. In the present survey small numbers were taken from 11 crops, namely, lucerne at Waipapa (24.2.59), Greenmeadows (9.2.59), Waipawa (9.2.59), and Martinborough (8.4.59); maize at Papamoa (4.3.59): chou at Masterton (8.4.59); and turnips at Maungaturoto (26.2.59), Ruakaka (2.3.59),

Silverdale (2.3.59), Waitoa (3.3.59), and Masterton (8.4.59). It occurred more frequently on lucerne and turnip crops. This species, which was commonly taken in pastures (Cumber 1959), may prove of some importance where crops are being held for seed production.

He. 62. *Cermatulus nasalis* (Westwood)

This species occurs in Australia and New Zealand (Tillyard 1926). Five nymphs were taken from chou at Waipawa on 9.2.59. Nymphs were observed at the time on a seedhead of *Amaranthus retroflexus* L. Tillyard records the species as attacking the pear slug (*Caliroa limacina* de Geer). It must be regarded as a beneficial species of minor importance.

He. 56. *Cuspicona simplex* Walk.

This species, which was first recorded from New Zealand by Spiller and Turbott (1944), is well distributed in the North Island, and occurs on the Three Kings Islands (Woodward 1953b). In the present survey one adult was collected from mangels at Ashhurst on 26.3.59. Woodward records this insect in association with solanaceous plants. The species is not important in fodder crops.

Fig. 7.—Distribution map for the pentatomid *Nezara viridula* He. 22.

He. 22. *Nezara viridula* (L.)*

This cosmopolitan species is distributed throughout the North Island, and occurs in the northern portions of the South Island. In the present survey it was taken in most North Island districts (Fig. 7) —128 adults and 28 nymphs being collected from lucerne (3 crops),

maize (17), chou (11), chou plus swedes (9), turnips (10), and swedes (1). It occurred in greatest numbers in Northland, irrespective of the type of crop. It also occurred in large numbers south of Auckland, e.g., turnips—Te Kuiti (22 adults) and Tauranga (9 nymphs); maize—Pio Pio (5 adults); chou plus swedes—Kotemaori (6).

Accounts of the habits of this species are given by Cumber (1949, 1951). Adults and nymphs were present for the duration of the survey (early February till early April).

It is interesting to note that maize crops in seven Northland areas showed only low infestations. It would seem that either this insect reaches pest proportions only in localised areas, or that its egg parasite, *Microphanurus basalis* Woll., is effective in local areas only.

N. viridula is a major pest of maize in Northland, occurring in such numbers as 11, 12, 15, 17, 20, and 26 per plant, and 7, 11, and 12 per cob. Damage to the plants is quite evident. Reddish-purple spots on the stems and cob sheaths mark the feeding punctures. Examination of cobs showed that the rostrums penetrated the sheath and pierced the grains. Many grains were split and many cobs malformed.

Chou was also found to suffer damage from this pest. At Waitoki, the insect occurred in recorded numbers of 4, 8, 11, and 20 per plant. The insects tend to congregate, groups of about 10 being quite common. Damage was evident as extensive puncturing on the stems. At Pakaraka, 28 adults and an empty egg-raft (no evidence of parasitism) were taken. Heavy infestations of chou and swedes were seen at Maungaturoto and Kotemaori, but no such infestations of swedes alone were encountered. Heavy infestations, with feeding on bulbs of turnips, were observed at Ruakaka and Te Kuiti. Lucerne did not appear to be heavily infested.

He. 53. *Oechalia schellembergii* (*Guérin-Ménville*)**

This species occurs in Australia and New Zealand (Tillyard 1926). In the present survey 5 specimens were collected from lucerne (Gisborne 10.2.59), maize (Mangamuka 23.2.59), chou (Puketona 23.2..59), mixed chou and swedes (Waihi 3.3.59), and turnips (Te Kuiti 20.3.59). An egg-raft was taken from chou at Waipawa on 9.2.59.

This is to be regarded as a beneficial species, since it is predacious on lepidopterous larvae etc.; but the numbers taken suggest that it plays a minor role in fodder crops.

He. 60. *Philapodemus australis* (Erichson)

This species occurs also in Australian and Oriental regions (Woodward 1953). A single adult was taken from lucerne at Waipawa on 26.3.59.

** Previously known as *O. consocialis* (Boisd.)

Discussion

Some 50 species of Hemiptera have been taken during this fodder crop survey. As was to be expected, many were taken in small numbers and probably represent casual associates as individuals of dispersions or associates of attendant weeds.

The following remarks concern the Hemiptera-Homoptera. The cicada species are casual associates, except in the case of pampas. Nymphal stages occupy several seasons, so that they are unlikely to be of importance in annual crops. *Erythroneura zealandica* would appear to feed on a large number of plants and must be regarded as a potential virus transmitter. The same potential exists for the remaining jassids and delphacids, but they were present in small numbers and on a limited number of occasions, as were *Thanatodictya tillyardi, Sephena cinerea, Scolypopa australis,* and the two psyllids. In the aphids, the majority of the species are casual associates. The importance of *Brevicoryne brassicae* and *Myzus persicae* in cruciferous crops is well known, however. It seems probable that *Rhopalosiphum padi* is of more importance in maize crops than is generally realised; perhaps its importance occurs during establishment, rather than at later stages.

Turning now to the Hemiptera-Heteroptera, there are a number of interesting considerations. The two *Nabis* species occurred commonly, and in their predacious capacities are doubtless useful biological control agents. *Calocoris norvegicus* did not appear in significant numbers, and perhaps prefers a cooler climate than is generally encountered in the North Island. Amongst the mirids the introduced species *Eurystylus australis* was consistently present, and warrants study to determine its precise importance. There is no evidence for important species among the lygaeids, although *Nysius huttoni* is known to be damaging to crops in some South Island areas. Amongst the pentatomids only *Nezara viridula* would appear to be important. It is still a serious pest of many crops in Northland—especially of maize and chou moellier—and warrants further attention in the biological control field.

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APPENDIX

SPECIES OF HEMIPTERA ASSOCIATED WITH FODDER CROPS

	Lucerne	Maize	Chou Moellier	Chou Moellier, Mixed Swedes	Turnips	Swedes	Mangels	Pampas	Rape	Rape, Swedes, Turnips, Mixed
Melampsalta muta								*		
Melampsalta sericea		*								
Erythroneura zealandica	*	*	*	*	*	*			*	
Deltocephalus sp. He 19		*								
Thamnotettix sp. He 13		*								
Thamnotettix sp. He 14					*					
Idiocerus sp. He 54 (?)							*			
undetermined jassid He 55	*									
Xestocephalus sp. He 11	*	*								
Delphacodes tasmani	*	*								
Delphacodes sp. He 4					*					
Thanatodictya tillyardi		*			*					
Sephena cinerea	*	*	*					*		
Scolypopa australis		*		*	*					
Ctenarytaina pollicaris	*									
Trioza sp. He 59				*						
Psylla sp. He 63			*							
Aulacorthum solani				*						
Brevicoryne brassicae			*	*	*	*			*	*
Capitophorus elaeagni	*			*	*					
Hyperomyzus lactucae		*								
Lipaphis erysimi			*	*	*					
Macrosiphum avenae					*					
Macrosiphum euphorbiae		*								
Macrosiphum sonchi		*	*	*	*	*				
Myzus ornatus	*									
Myzus persicae		*	*	*	*	*				*
Rhopalosiphum maidis		*	*							
Rhopalosiphum padi		*	*		*	*				
Rhopalosiphum splendens			*		*					
Nabis capsiformis	*	*	*	*	*	*				
Nabis maoricus	*		*		*					
Calocoris norvegicus			*							
Deraeocoris maoricus		*			*					
Eurystylus australis	*	*	*	*	*	*	*		*	*
Halticus tibialis	*	*			*					
Lygus sp. He 50		*								
Megaloceroea reuteriana		*								
Lyctocoris campestris		*								
Poronotellus whitei		*								
Nysius huttoni	*		*							
Pachybrachius nigriceps		*								
Rhypodes clavicornis (?)	*									
Antesia orbona					*					
Dictyotus caenosus	*	*	*		*					
Cermatulus nasalis			*							
Cuspicona simplex							*			
Nezara viridula	*	*	*	*	*	*				
Oechalia consocialis	*	*	*	*	*					
Philapodemus australis	*									

Bacillus thuringiensis BERL. AS A POTENTIAL MEANS OF CONTROL OF CABBAGE WHITE BUTTERFLY (*Pieris rapae* L.), DIAMOND-BACK MOTH (*Plutella maculipennis* CURT.), AND SOME OTHER LEPIDOPTERA

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Summary

The microbial control of insects, though not new, has developed rapidly over the last decade by adopting many of the principles of biological and chemical control. When, late in 1959, an experimental sample of a commercial wettable powder containing 3,000,000,000 viable spores per g of a bacterium, *Bacillus thuringiensis* Berliner, became available, small-scale trials were carried out to test the efficacy of the micro-organism against *Pieris rapae* L., *Plutella maculipennis* Curt., and other lepidoptera.

The present paper records these trials, which showed that, while the bacillus had no effect on *Brevicoryne brassicae* L., syrphid larvae, and hymenopterous parasites, it was most effective against larvae of *P. rapae* and *P. maculipennis,* and was also effective against the larvae of *Plusia chalcites* Esper., *Agriophora coricopa* Wlk., and *Tortrix* sp.

Introduction

The recent rapid development of organic insecticides, many of them highly toxic to warm-blooded mammals and man, which may leave harmful residues on foodstuffs, has led to the introduction of legislation in various countries setting out permissible maximum tolerances. As more and more has been learned of the toxic hazards of these substances, some of these permissible residues have been reduced. On the other hand, the continued use on a large scale of some of them has caused natural selection to produce resistant strains, so that these insecticides are no longer as fully effective against the particular pest species. At the same time, parasites and predators of pest insects in home and garden have been killed off with the pest, thus upsetting the natural biological balance in given ecological situations.

As a result, attention has been turned to new ways of killing insects which do not possess these undesirable features and yet which produce an economic control of pest species. Microbial control has proved a promising method. So far the best results have been obtained with spore-forming bacilli on a wide range of lepidopterous insects, particularly with wettable powders and dusts containing viable spores of *Bacillus thuringiensis* which can be prepared commercially.

The earliest use of natural parasites or predators, usually other insects, to achieve successful biological control of insect pests appears to have occurred in the eighteenth century. The growers of date

palms in Arabia every year brought colonies of predatory ants from the mountains to attack and control vast swarms of ants which seriously attacked their date palms (Nicol 1943).

In 1800, in his "Phytologia, or the Philosophy of Agriculture and Gardening" (page 356), Erasmus Darwin suggested that insect pests might be controlled by carefully preserving, during the winter months, the eggs of certain other insects which preyed on the pests, and then disposing of these eggs on peach and nectarine trees in early spring.

The real beginning of biological control, however, followed the introduction of the Australian ladybird beetle (*Rodolia cardinalis* Muls.) into California in 1888 (Koebele 1890). This beetle was used against Cottony Cushion Scale (*Icerya purchasi* Mask.), which had been accidentally introduced into California earlier. The scale was reduced to a minor status within five years of the introduction of the ladybird beetle (Sweetman 1958).

The recent development of insect pathology (Steinhaus 1956) focused attention on the use of micro-organisms as a new way of killing insects. Although it has developed rapidly over the last decade, this idea is not new; references to it in bee and silk worm cultures were made long ago by such ancient writers as Aristotle (384–322 B.C.) and early Chinese authors. The historical aspects have been fully described elsewhere (Steinhaus 1956).

Microbial control has developed from this background, and, over the past 10 years, has advanced rapidly by adopting many of the principles of biological and chemical control to which it is closely related (Steinhaus 1949). This technique employs such micro-organisms as pathogenic viruses, Rickettsiae, bacteria, nematodes, and protozoa which cause disease in insects. Stimulated by the recent development in insect pathology, attention was focused on these micro-organisms as a means of control. As a result, virus diseases have been used with some success (Glass 1958). Viruses, however, can only develop and multiply in living cells, and, until techniques of tissue culture are developed, this requirement makes it difficult to produce large quantities on demand.

Rickettsiae, fungi, nematodes, and protozoa have not been extensively used because of their dependence on environmental factors (Fox and Jacques 1958).

On the other hand, some of the bacteria that attack insects can be grown outside their hosts, thus permitting the production of large quantities. This large-scale production is particularly applicable to spore-forming bacteria; the subject has been reviewed by Tanada (1959).

In 1915 Berliner described a new species of spore-forming bacterium (*Bacillus thuringiensis* n. sp.) derived from diseased flour moth larvae (*Ephestia kuhniella* Zell.) in Germany.

It had been known for many years previously that a number of lepidopterous species were killed by bacteria of this type, but the cause of death was not known. As a result of recent research much more

is now known of the mode of action of these spore-forming bacteria. The caterpillars ingest the spores along with foliage consumed. The spores germinate in the gut, and the bacteria invade all parts of the insect's body, causing the death of the host. The caterpillars become inactive almost as soon as the infection sets in, and infected individuals seem to lose all co-ordination and may fall from the plant unless glued on by dried discharge. The insects turn dark and dry up. Most bacterial infections resemble each other in their symptomology, and similar symptoms may be elicited in different hosts by either *B. thuringiensis* or by other species of bacteria (Tamashiro and Tanabe 1959).

In this way a new category of insecticides–"microbial insecticides"–emerged, but there are as yet only one or two examples. Only one of these "microbial insecticides" has been commercially produced; its active principle is the viable spores of *Bacillus thuringiensis* Berliner. This product has been granted a temporary exemption from a residue tolerance in the United States for application to a wide group of food and forage crops. It appears promising for use on maturing crops such as cruciferae and lettuce, and against a number of lepidopterous larvae, as well as one or two Coleoptera, because of its apparent harmlessness to warm-blooded animals.

EXPERIMENTAL PROCEDURE

Field Trial on Cabbage White Butterfly and Diamond-back Moth

A small quantity of commercial wettable powder containing 3,000,000,000 viable spores of *Bacillus thuringiensis* per g became available late in 1959. This permitted a small-scale trial in January 1960 on a garden plot of cabbages that was severely infested with Cabbage White butterfly (*Pieris rapae* L.) and Diamond-back Moth (*Plutella maculipennis* Curt.).

In this trial the hearts of 18 cabbages were sprayed with the powder at the rate of 6 lb to 100 gal water plus a small amount of wetting agent (the sodium salt of alkyl naphthalene sulphonic acid and pine oil) to ensure thorough wetting of the leaves. One cabbage was left unsprayed. The spray was applied to the hearts with a hand atomiser giving a very fine particle size. Application was made in the early afternoon of 24 January. and very heavy rains and high winds followed that night. Two days later, on 26 January, an examination of the cabbages revealed many freshly dead caterpillars of both species of moth, while others were dying. Four days later, on 28 January, all caterpillars of both species were dead.

The cabbages were again examined on 6 February, at which date the sprayed plants remained free of both species of caterpillar. This examination revealed that cabbage aphid (*Brevicoryne brassicae* L.) was not affected by the spray. At this date the unsprayed cabbage was infested with half grown caterpillars of Cabbage White butterfly (*Pieris rapae* L.). This result is in agreement with that obtained on the same species of butterfly at Geneva, U.S.A., in which 0.3 lb per acre of a preparation containing 100,000,000 spores per g proved

superior to a suspension of the granulosis virus, *Bergoldia virulenta* (McEwen and Hervey 1959). This result also compares favourably with the results obtained in Hawaii by Tanada (1956), and Grigarick and Tanada (1960). The trial also suggests that, because of the very much greater number of viable spores per g, concentrations less than that applied in this experiment might be equally effective.

Seventeen days after application of the original spray, reinfestation of the first cabbage with *P. rapae* was observed; on 11 February the cabbages were therefore again sprayed at the same rate. On this date caterpillars of *P. maculipennis* were hatching and newly laid eggs of *P. rapae* were abundant on all cabbages. All caterpillars were again dead and dying two days after application and only dead caterpillars could be found four days after spraying. Dying caterpillars collected two days after application were removed to the laboratory to permit examination of the tissues by microscope. Smears of the body fluids of these dying caterpillars, stained with Ziehl-Nielsen's carbol fuchsin and counter-stained with Löffler's alkaline methylene blue, showed the fluids to be heavily infected with the bacillus and revealed large numbers of spores. (Fig. 1.) *Brevicoryne brassicae*, syrphid larvae, and hymenopterous parasites remained unaffected by the bacillus.

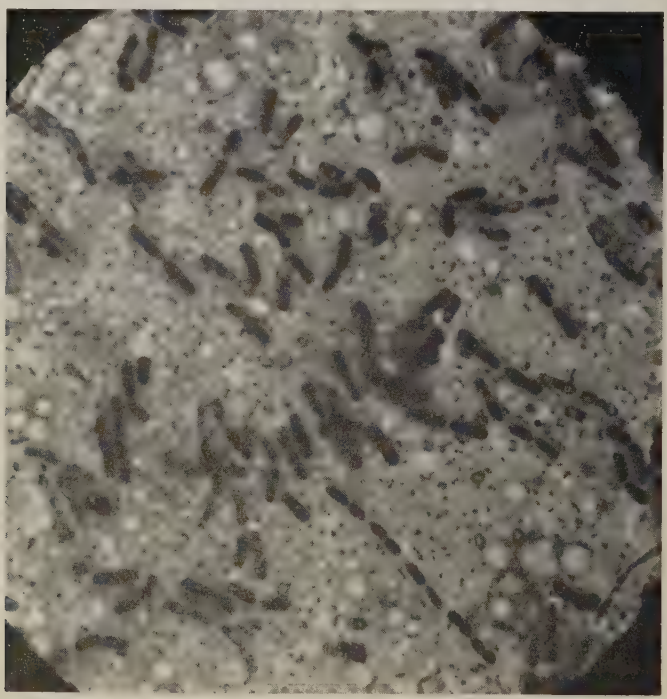

Fig. 1.—Smear of the body fluids of the larva of *Pieris rapae* L. two days after application of spray, showing rods of *Bacillus thuringiensis* Berl.

On 9 March, 27 days later, reinfestation with a few larvae of *Pieris rapae* was observed, at which time the cabbages were heavily infested with eggs. At this time the hearts were still clean and uninjured. During these 27 days butterflies continued to oviposit freely on the cabbages, which remained clean. Very little rain fell during this period to remove any traces of bacilli remaining from the last treatment of 11 February.

Caterpillars of *P. rapae* were collected on 15 March, 33 days after application, when damage was beginning to show again on the treated cabbages. Smears of the body fluids and tissues of these caterpillars, prepared as before, failed to reveal the presence of any bacilli. By 22 March the first of these caterpillars had reached the final instar and was feeding normally. A third and final application of spray mixture was made on 23 March, 41 days after the last application, and no further applications were necessary before harvest.

Tests on Silver Y Moth, Olearia Moth, and Leafroller

Three additional trials were made to test the efficacy of the bacillus against larvae of Silver Y Moth, *Plusia chalcites* Esper., Olearia Moth, *Agriophora coricopa* Walk., and Leafroller, *Tortrix* sp.

Bean leaves heavily attacked by caterpillars of *P. chalcites* were sprayed with a suspension of the bacillus spores at the rate of 6 lb/100 gal as already described for cabbage butterfly. Within two days caterpillars were dying in all instances except the first instar larvae; three days after application all caterpillars were dead. Smears made two days after application, immediately following the death of the first caterpillars, revealed only a very occasional bacillus. At the end of the third day, however, the smears showed that all caterpillars were very heavily infected with large numbers of sporulating bacilli.

In the second trial a bush of *Olearia Fosteri*, heavily attacked by Olearia moth, *A. coricopa,* was sprayed with bacillus spores in a way similar to that described for the other three species of moths. Caterpillars began dying within four days of application, and smears at this time showed the presence of rods of *B. thuringiensis*. Twelve days after application the smears showed that the body tissues were heavily infected with large numbers of sporulating bacilli.

In each case the bacillus recovered was morphologically the same as that recovered from larvae of *P. rapae* and *P. maculipennis*, where the rods measured 3 μ by 1 μ.

Late in the season a small field trial was carried out on a row of cabbages infested with leafroller, *Tortrix* sp., which was invading the cabbages from an adjacent row of poplars in the Hutt Valley. On 3 May the cabbages were sprayed at the same rate as before. Six days after application 75% of the caterpillars were dead and dying. Smears of the body tissues at this time revealed vast numbers of spores of the bacillus. Fourteen days after application all larvae were dead.

Results

The results suggest that this microbial method is a very promising means to control both *P. rapae* and *P. maculipennis* on cabbage when used at 16-day intervals. However, further work is necessary to establish dosage levels, best methods of application, and proper timing of applications. Since aphids are unaffected it may also be necessary to investigate bacillus-aphicide combination treatments to protect the crop adequately, and to discover whether natural enemies of these pests build up in bacillus-treated areas.

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SOME PRELIMINARY OBSERVATIONS ON FACTORS AFFECTING THE TEMPERATURE AT THE SKIN SURFACE OF THE NEW ZEALAND ROMNEY SHEEP

I. THE ESTIMATION OF SKIN TEMPERATURE

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Summary

Skin temperatures at various positions on four sheep have been studied in order to investigate the possibility of relating cutaneous blood flow to certain facets of wool growth. While temperature differences were found to occur between sheep, and between different body positions on an individual sheep, and measurements were influenced by many other factors such as the ambient temperature, diurnal variation, the effects of handling and the insulation of the skin. Since the individual sheep varied in their diurnal trends and handling responses, the confounding influence of these responses should be taken into account in any attempt to correlate skin temperature and wool growth.

The skin temperatures of two groups of 20 sheep were also measured under field conditions, and it was found that repeatable measurements could be obtained at the time of day when skin temperatures were the greatest.

Introduction

Differences in wool growth on the face of the New Zealand Romney ewe are associated with differences in both the body growth of the lamb and the fertility of the ewe (Cockrem *et al.* 1956; Inkster 1956). As part of an investigation of this association the factors which could limit wool growth on the face are being studied. As wool-producing follicles are present on the face of both woolly and open-faced sheep (Cockrem unpublished), the limiting factor is likely to be physiological rather than anatomical. The blood supply to the follicle could be such a limiting factor and one of the few methods of estimating differences in blood supply to the skin area is by means of the temperature of the skin. The use and interpretation of such a method of measurement have been discussed by Burton (1948) and Hertzman (1953).

The present study was made in order to determine possible factors which should be taken into account when determining skin temperature and to develop a technique whereby a sufficient number of sheep could be measured in order to estimate possible relations of skin temperature with wool growth.

Methods

The sheep used for the pen trials were four aged Romney ewes. Three were open-faced (Nos. 7, 18 and 21) and one woolly-faced

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(No. 66). The three open-faced animals had had the right inferior cervical sympathetic ganglion and a portion of the cervical sympathetic nerve removed 10 months previously. Six Romney ewes formerly employed for experiments with thyroxine (Kirton *et al.* 1959) were also used for some preliminary temperature measurements.

The sheep were kept in separate pens in a partly open shed, the skin temperature readings being made without removing the animals from the pen. Initially the readings were made with a copper-constantan thermo-couple which had been standardised by immersion in water at different temperatures. Later readings were made with a Thermophil,* a thermistor type of instrument giving a direct reading of the temperature of a surface. Air temperatures were recorded for each series of skin temperature measurements.

The collection of data was not entirely systematic as methods were varied to overcome disadvantages as they were encountered. Initially, measurements were taken on the mid-side position without clipping the wool. However, it was necessary to clip the wool or kemp in order to obtain contact with the skin on the poll (position 1), and on the side (position 2) and centre (position 3) of the nose. A reading was also taken on a clipped area below and posterior to the eye (position 4).

All the thermo-couples were tested before use on the surface of a poly-ethylene bag filled with warm water. The surface temperature of this bag was measured with a mercury thermometer and those couples which failed to give a reading comparable to that obtained by immersion were discarded. Similar readings were obtained for the thermo-couples and Thermophil when they were checked side by side on the bag or on the skin of the sheep.

A reading was taken at each of a series of positions, followed immediately by a series of repeat measurements; positions were measured in a different random order, with the restriction that readings all started and finished on the face positions. Thus about one to four minutes elapsed between the original and the duplicate reading on any one site. When possible, four sets of readings were taken in a day so that, on any one day, the variation between the sheep, the time of day and the repeat readings, could be analysed.

For the field trials two groups of Romney ewe hoggets were selected at random and the measurements were made in May and October. The animals were brought to the yards at 8.30 a.m. and were measured several times during the day, being held in the yards in the interval. About one hour was needed to measure one group of 20 to 25 sheep and this was found to be the maximum time over which comparisons within the group could be made without sheep differences being confounded with those arising from the diurnal trends in skin temperature. Table 1 shows the times at which the readings were taken for each group.

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TABLE 1. THE MEASUREMENT STARTING TIMES FOR THE FIELD TRIALS IN MAY AND OCTOBER

Reading	Group A		Group B			
	1	2	1	2	3	4
May	9.20 a.m.	2.20 p.m.*	10.45 a.m.	11.30 a.m.	1.45 p.m.	3.30 p.m.
October	11.30 a.m.*	3.00 p.m.*	—	—	2.00 p.m.*	—

* Two repeated readings made.

In May immediately repeated readings were taken on the same position in order to test for possible differences arising from variations in pressure on the probe. In October the repeated readings were those taken on the two sides of the nose. For Group A half the animals had the left side taken first in the morning and the right side first in the afternoon and the remainder vice versa. In this way position effects could be compared with possible changes occurring during the time that the measurements were taken.

The Thermophil was used for all the field measurements with a more sensitive probe than that used in the pen trials. An area about 4 × 4 cm on the side of the nose was clipped clear of fibres the previous day and measurements were made in the centre of this patch. The pressure applied to the probe was just sufficient to make a slight mark on the skin.

RESULTS

PEN TRIALS

Accuracy

Table 2 shows the percentage of variation arising from sheep, time of day and repeated measurements for position 2* and 4. It was found that little extra information was obtained from positions 1 and 3, in that the readings were related to the remaining positions, but were more difficult to take because of underlying bony structure. The readings made under a full fleece on the mid-side position were almost constant. The data from the thermo-couple and from the Thermophil were obtained on separate days.

It can be seen that little of the variation was directly the result of a consistent bias of the repeated readings, although for position 4 the sheep-repeat interaction indicated that differences in repeated readings occurred for individual animals. This did not always occur, but when it did the difference was always in the same direction for a particular animal. It was most marked on the centre of the nose and this position was therefore not used for subsequent trials.

The standard deviation from the triple interaction indicates that, with both the thermo-couple and the Thermophil, the majority of

* Unsympathectomised side.

TABLE 2. The Analyses of Variance of Repeated Thermo-couple and Thermophil Readings

Source of Variation	Estimated Components of Variance as Percentages†			
	Side of Nose (Position 2)		Cheek (Position 4)	
	Thermo-couple	Thermophil	Thermo-couple	Thermophil
Sheep (S)	61.9**	35.8*	0.0	1.6
Repeats (R)	0.0	0.0	0.0	5.4
Time (T)	22.4**	28.5	13.7	18.2
S × R	0.3	0.4	36.5**	7.7*
S × T	13.2***	30.6***	36.4*	42.8***
R × T	1.2*	0.0	0.0	10.7*
S × R × T	1.0	4.6	13.4	13.5
Standard deviation:				
from S × R × T	0.626	1.492	0.577	0.343
from total	5.732	6.306	1.241	0.889
n	32	30	32	30

† The tests of significance are those from the ratios of mean squares (F), and where interactions were significant the main effects were tested against the largest relevant interaction.

* P less than 0.05
** P less than 0.01
*** P less than 0.001

their repeated readings are within 1°F under the conditions of these trials. The standard deviation of 0.34°F and the significant sheep-time interaction in position 4 suggested that the biological variation of the sheep, not the accuracy of the Thermophil, was likely to be the factor limiting more accurate estimation of individual skin temperatures. Independent trials of the Thermophil under less exacting conditions on the skin of the human leg indicated an accuracy shown by a standard deviation of 0.2°F for repeated readings.

Two other points are shown by Table 2: first, that, among a group of only four sheep, it is possible to detect differences in the temperature of the skin on the side of the face; and, secondly, the importance of the time of day at which the readings are taken. Not only is there a marked diurnal trend in skin temperature on these positions, but there is also a significant interaction between sheep and time, which indicates that there are differences in this diurnal trend between animals.

Position and Time Differences

The result of an analysis of position differences is shown in Table 3, and the mean skin temperatures at these positions are shown in Table 4. An analysis for the side of the nose (position 2) for readings at various times on two days is also shown. These measurements were made with the thermo-couple and the means of two repeated readings made at each time were used for analysis.

TABLE 3. THE ANALYSIS OF VARIANCE IN SKIN TEMPERATURE BETWEEN POSITIONS AND BETWEEN DAYS FOR THE NOSE POSITION 2

Estimated Components of Variance as Percentages*

Source of Variation	Between Side of Nose, Poll, Mid-Side and Cheek	Source of Variation	Between Days for the Side of Nose
Sheep (S)	2.9	Sheep (S)	59.0***
Time (T)	4.3	Time (T)	14.2**
Position (P)	34.4*	Day (D)	8.6*
S × P	46.6***	S × D	0.2
S × T	2.7*	S × T	0.0
T × P	5.3**	D × T	5.8
S × P × T	3.9	S × D × T	12.1
Standard deviation	0.680	Standard deviation	2.593
n	48	n	24

* See note below Table 1.

TABLE 4. THE MEAN SKIN TEMPERATURES IN DIFFERENT POSITIONS (°F)

	Nose Centre	Nose Left Side	Below Eye	Side of Neck	Poll	Mid-Side*
Sheep No. 7: Day 1	77.8	81.8		96.6		
Day 2		65.1	97.8		95.3	99.7
18: Day 1	89.9	92.2		97.9		
Day 2		95.9	98.1		96.2	99.6
21: Day 1	91.2	92.5		97.8		
Day 2		95.7	98.9		97.1	99.5
66: Day 1	84.2	91.7		97.2		
Day 2		97.4	97.6		96.5	99.0

* Under full fleece, all other areas were clipped.

It can be seen that the position effects were both important and significant and that they were not constant between sheep, as was shown by the significant sheep-position interaction. For these reasons the means in Table 4 are given for the individual sheep. It can be

TABLE 5. SKIN TEMPERATURES IN THE CENTRE OF THE NOSE FOR THE SIX SHEEP USED FOR PRELIMINARY TRIALS (°F)

Sheep	1*	2	3	4*	5	6*
Day 1	70.1	86.6	84.5	78.1	90.3	91.3
Day 2	79.8	85.0	82.5	80.8	87.8	88.5
Day 3	68.5	73.0	70.0	75.5	90.0	92.7

* Treated with thyroxine in a previous experiment.

seen that differences in skin temperature for position 2 can be considerable and can also vary from day to day. The variation between sheep and between days can also be seen in the readings on the nose for the six sheep used in the preliminary trials (Table 5).

Daily Trends and the Effects of Ambient Temperature (position 2)

The differences between days and the high triple interaction shown for position 2 in Table 3 may have resulted from variations in the ambient temperature. Fig. 1 shows the daily range of skin temperature on position 2 for the four sheep on two separate days. It can be seen that the readings increased over the day and were also higher for all sheep on the second day. Sheep 7 had consistently lower temperatures than the others.

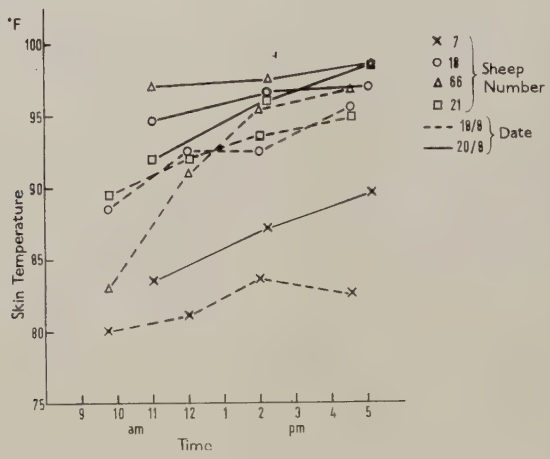

Fig. 1.—The daily range of skin temperature on the side of the nose for individual sheep on two separate days.

The differences between days were examined by relating the mean skin temperature for the day with the mean air temperature on that day. The regression was calculated as the average one within sheep to allow for the differences between animals. The four days' readings available gave a regression coefficient of 0.37 ± 0.106 ($P < 0.01$ 12 d.f.), i.e., a rise in ambient temperature of 1°F was associated with a rise of about 0.4°F in skin temperature. This was consistent for each of the four animals and suggests that ambient temperature was one source of the variation between days.

The Effect of Shearing

The sheep were in full fleece for the first part of the trial and very little variation was found in the skin temperature of the main body areas. This may well have resulted from the insulation provided by the fleece. However, as complete shearing of the sheep can alter its physiological status, the possibility of overcoming the insulation effect of the wool, by making measurements on a shorn patch, was therefore

investigated. Two sheep were completely shorn, while the other two had a patch about eight inches square shorn on one side only. Two days later these two animals were also completely shorn.

TABLE 6. THE EFFECT OF SHEARING ON SKIN TEMPERATURE (°F)

	Day 1 (Ambient Temp. 58°)				Day 3 (Ambient Temp. 59°)			
	Shorn		Unshorn		All Sheep Shorn			
Sheep	66	7	18	21	66	7	18	21
Left side of nose	71.3	78.2	90.3	88.1	74.7	80.8	79.0	77.1
Mid-side	79.6	85.4	98.0	97.0	92.9	90.3	93.8	89.3
Centre of shorn patch			89.6	93.1				
Shoulder	95.9	95.0	—	—	95.6	93.8	94.3	91.7
Britch	93.8	92.2	—	—	95.6	93.3	96.0	92.0
Average previous unshorn readings:								
Side of nose	94.9	86.6	—	—	—	—	—	—
Mid-side	98.5	99.2	—	—	—	—	—	—

Table 6 shows the skin temperatures for three body positions and for the side of the nose before and after these various treatments. The shoulder and britch showed readings similar to those of the mid-side before shearing. Table 7 shows the variation in skin temperature over the shorn patch.

TABLE 7. MEAN READINGS ($n = 3$) IN DIFFERENT POSITIONS ON SHORN PATCH FOR SHEEP 18

Position	Anterior in Fleece	Anterior Edge	Centre	Posterior Edge
Skin Temperature (°F)	97.9	96.7	90.4	97.0

Complete shearing lowered the skin temperature considerably, the reduction being more marked for the nose and mid-side positions than for the shoulder and britch. However, the mid-side position showed some recovery, but this was not able to be followed after the third day because of a confounding effect of rising ambient temperature. The clipping of the patch resulted in lowered skin temperatures in the centre of the patch, but did not affect the unshorn areas, either immediately adjacent or on other body positions.

FIELD TRIALS

Table 8 shows the results of analyses of variance of the repeated measurements of skin temperature in May for Group A at 2.20 p.m. and for the one measurement at four different times for group B.

TABLE 8. The Analyses of Variance for Measurements in the Field in May

Group A. Repeated Measurements at 2.20 p.m.

Source	d.f.	Mean Square	Variance Component %
Repeats	1	0.253	0.0
Sheep	22	25.892	91.8
Remainder	22	1.535	8.2

Standard deviation 1.24°F

Group B. One Measurement at Four Times

Source	d.f.	Mean Square	Variance Component %
Time	3	28.279	9.3
Sheep	22	21.645	37.7
Remainder	66	5.634	53.0

Standard deviation 2.37°F

For Group A the lack of any difference in the repeated measurements and the small interaction between sheep and repeated measurements suggest that variation from the method of measurement was unimportant.

For Group B a major source of variation was an interaction between the sheep and the time of the measurement, i.e., the sheep did not fall in the same order at each time. This could have arisen from different diurnal trends in temperature between the sheep.

Table 9 shows the correlations between the skin temperatures of individual animals taken at the different times. It appeared that readings made before 11 a.m. were not repeatable later in the day. However, readings made between 11.30 a.m. and 3.30 p.m. (Group A October, Group B May) were inter-related, although the two extreme times showed only a low correlation.

A further analysis was made of the readings obtained in October, when the ambient temperature was the same as that in May. Readings made on the left and the right side of the nose were available. No difference was found to be associated with whichever side of the nose was measured first; the data were therefore analysed as first or second readings, ignoring the side of the nose. Table 10 shows the results from the analyses of variance for Group A for sheep, time and the repeated readings on each side of the nose. Results of analyses of the afternoon readings only are also given for Groups A and B. Data from two sheep were omitted from the analysis of Group B, the first because of a skin temperature drop of over five degrees while the readings were being taken, which occurred for this sheep only, and the second set of data because of erratic readings in both May and October resulting from the violent behaviour of the sheep.

TABLE 9. THE CORRELATIONS BETWEEN MEASUREMENTS MADE AT DIFFERENT TIMES FOR THE FIELD TRIALS (ALL THE COEFFICIENTS ARE POSITIVE)

Group A

		May		October
		9.20 a.m.	2.20 p.m.	11.30 a.m.
May	2.20 p.m.	0.30*	—	—
October	11.30 a.m.	0.29	0.21	—
October	3.00 p.m.	—	0.41	0.53†

* 21 d.f. non-sig. † 18 d.f. P = 0.02 Remainder 17 d.f. r = 0.46 at 5% level.

Group B

		May			October
		10.45 a.m.	11.30 a.m.	1.45 p.m.	2.00 p.m.
May	11.30 a.m.	0.36	—	—	0.05
May	1.45 p.m.	0.32	0.56	—	0.21
May	3.30 p.m.	0.33	0.43	0.52	0.27

May with October 19 d.f. Remainder 21 d.f. r = 0.42 at 5% level.

For Group A the interaction between sheep and time was again important, but there was little variation from repeated measurements. When measurements taken at the one time were analysed, a high proportion of the variation was associated with sheep, and the standard deviation of ± 1°F from the error term was similar to those values obtained in the pen trials.

For Group B the standard deviation was higher (± 1.5°F), but the lesser variation of the sheep may in part arise from the lower total variance of this group.

The correlations between May and October readings are shown in Table 9. The only correlation suggesting a real relationship is that for Group A at comparable afternoon times. However, as Group A readings were the more repeatable and had the greater overall variation, then the significant correlation suggests that the methods used can give meaningful values. Furthermore, the values obtained were characteristic for a particular sheep over at least six months.

DISCUSSION

The accuracy of a technique should be considered in relation to the size of differences having biological meaning and the variation shown by the animals to be measured. For this reason, results have been pre-

TABLE 10. THE ANALYSES OF VARIANCE FOR MEASUREMENTS IN THE FIELD IN OCTOBER

Group A

All readings

Source	d.f.	Mean Square	Variance Components %
Time (T)	1	21.218	3.5
Repeats (R)	1	0.578	0.0
Sheep (S)	19	22.154	45.9
R × T	1	2.312	0.2
S × T	19	7.527	30.1
S × R	19	1.237	0.0
Remainder	19	1.899	20.3

Standard deviation 1.38°F

11.30 a.m. only

Source	d.f.	Mean Square	Variance Components %
Sheep	19	17.572	78.1
Repeats	1	2.601	0.2
Remainder	19	2.146	21.7

Standard deviation 1.47°F

3.00 p.m. only

Source	d.f.	Mean Square	Variance Components %
Sheep	19	12.110	84.9
Repeats	1	0.289	0.0
Remainder	19	0.990	15.1

Standard deviation 0.99°F

Group B

Source	d.f.	Mean Square	Variance Components %
Sheep	20	7.702	55.8
Repeats	1	2.881	0.7
Remainder	20	2.159	43.5

Standard deviation 1.47°F

sented in the form of components of variance, although these themselves are estimates and liable to error.

However, a clear pattern of a high proportion of variation between sheep, compared with that between repeated measurements on the same position on the same sheep, was found for both the pen and the field trials. The possibility of the sympathectomy increasing the variation between sheep in the pen trials seems unlikely in the light of the similarity of results to those obtained in the field trials. The methods used therefore appear to be satisfactory for estimating skin temperatures where variation of the order of 2–3°F exists between sheep. This is true

for the face region; but the methods would not be satisfactory for detecting small differences, and other methods would be required where such differences were likely to be of biological value. However, as differences of 1°F can occur between the right and left sides of a normal animal (Burton 1948; Beakley and Findlay 1955), differences between animals of less than this value might be difficult to interpret.

An important source of variation was that from ambient temperature and the diurnal trend. As the diurnal trend differed between sheep it appeared that, for studies of the type described in this paper, it was more important to have sufficient sheep and a knowledge of the diurnal trends than to have a very accurate method of measurement. The change of 0.4°F on the face, with a 1°F change in ambient temperature, agrees well with the value of 0.6°F found by Blaxter *et al.* (1959) for the ear of the sheep.

Because of these sources of variation, when pen space is limited, the use of field techniques where 20 or more animals can be measured with reasonable accuracy at the appropriate time of day is suggested. Such data could then be used with those from more detailed readings on a few penned sheep.

Experiments are still required to check the relation of skin temperatures measured by these methods with the actual blood supply to the skin. Fetcher *et al.* (1949) showed that a high rate of heat transfer from the skin was necessary for skin temperature to be closely related to blood flow. The measurements made under a full fleece clearly did not satisfy these requirements and extensive shearing would change the physiological status of the sheep. This could be overcome by clipping a patch about eight inches square which lowered the skin temperature of the patch but not of other regions. This suggests that the heat transfer was increased without altering the physiological status of the animal.

For the field readings the relations between measurements taken in May and October, although small, suggested that some real property of the sheep was being measured by facial skin temperature. If such readings as those made in the field were found to be related to the amount of wool growth on the face, then this would be suggestive of a relation between face cover and the blood supply to the face. A more direct test of the hypothesis would be the use of treatments which changed the blood supply to this region, followed by measurements of possible changes in wool growth. The second paper of this series will present the effects on skin temperature arising from various treatments designed to increase or decrease the blood flow to the face.

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SODIUM METABISULPHITE AS AN ADDITIVE IN SILAGE MAKING

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Summary

The chemical composition, nutrient losses, calculated feeding value, and palatability of silages made from chopped herbage at two stages of growth, each with and without sodium metabisulphite, were compared.

All silages had low volatile fatty acid contents. Mean pH values of untreated silages approached 4.0, while those silages treated with sodium metabisulphite were higher. Metabisulphite silages had a much higher carotene content than their controls.

With silage made from immature herbage, metabisulphite reduced the loss of dry matter and organic matter, but not of crude protein or minerals. With more mature herbage metabisulphite had little effect on losses.

Metabisulphite slightly increased the digestibility of the crude protein and crude fibre fractions in both the immature and more mature silages, but had little effect on the other constituents. In consequence, treated silages had slightly higher calculated feeding values than their controls.

Dairy cows, given a free choice of treated and untreated silages, showed a significant preference for the former, irrespective of stage of maturity of the ensiled crop. Nevertheless, untreated silages, when fed alone, were readily consumed by the same animals.

It was concluded that the small saving in digestible nutrients, which resulted from metabisulphite application to immature chopped herbage, ensiled under good conditions, did not justify the cost and trouble of using the additive and that no advantage was gained by applying the salt to mature material.

Introduction

A number of workers have reported that the treatment of immature herbage with sodium metabisulphite reduced storage losses and improved the quality of the resultant silage (Cowan *et al.* 1953, 1956; Bratzler *et al.* 1956; Murdoch and Holdsworth 1958).

Their results seemed sufficiently encouraging to warrant research under New Zealand conditions, since nutritive losses in silage made throughout the country are high (Sill and Sears 1942). Accordingly, trials were conducted in which a grass and legume sward was cut at two contrasting stages of growth and ensiled in concrete experimental silos, with and without addition of metabisulphite. The silages were compared as to their chemical composition, the nutrient losses incurred during storage, and their nutritive value.

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Experimental Methods

Ensiling Technique

To provide the herbage, two one-acre blocks were selected at random within a six-acre field of permanent pasture on high-fertility land.

The botanical composition of the sward was determined from 10 samples of turf taken prior to the cutting of each block. Immature material was harvested, starting on 31 October when the pasture was 12-15 in. in height and still making leafy growth. The more mature material was ensiled 24 days later from the second block when the herbage was approximately 18 in. in height and the rye-grasses were headed but still in the pre-bloom stage. The crops were cut with a mower, picked up and chopped into 4-6 in. lengths with a forage harvester and transported to the silos as rapidly as possible to avoid wilting.

The concrete silos used had a capacity of four tons of chopped green material. They were rectangular, with rounded corners, and in each a 6 in. layer of stones was placed to facilitate drainage and the collection of exudate from earthenware pipes leading from the base.

At it was thought that the chopped immature leafy herbage with its high clover content might pack very tightly and produce a sour unpalatable silage, the "low temperature process" as advocated by Watson (1939) for untreated material of this type was used for the immature control silage. The filling of the other three silos was rapid and continuous, a process recommended for mature herbage (Watson 1939; Barnett 1954) and one which should prove satisfactory when a sterilising agent is employed as an additive. During the first day of operations, the silo with the untreated immature herbage was filled to one-third of its capacity and the mass left until the following day. On the second day both silos were filled rapidly, loads being alternated between the silos. Each was topped up on the third and sixth day and thereafter sealed by a layer of sisalkraft paper and an 8 in. layer of soil. With the more mature material, filling was completed within three hours and the silos were sealed immediately. All silos were roofed.

Green material was weighed in lots of 50 lb, spread evenly in the silos and well consolidated by trampling. Sodium metabisulphite was applied as a finely ground powder at the rate of 10 lb per ton of herbage. To ensure even distribution of the salt, 200 g portions were sprinkled evenly over each 100 lb of fresh herbage as ensiled and green material immediately added to prevent possible loss of sulphur dioxide. Thermo-couples were placed in the centre of each silo at heights of 2 ft and 4 ft from the base.

Duplicate samples of herbage, made up of sub-samples from each 50 lb lot, were taken for dry matter determination and subsequent chemical analysis after each 1,000 lb of material were ensiled. Samples were dried in a forced draught electric oven at 150°F, finely ground in a laboratory mill and stored in air-tight containers. Determinations of

crude protein, organic matter, and mineral matter were conducted in duplicate on representative laboratory samples and the weights of these constituents in each silo calculated.

Determination of Nutrient Losses

Daily measurements of exudate from the ensiled crops were recorded. A 200 ml sample was taken at each collection period and analysed. The losses of dry matter, organic matter, crude protein, metabisulphite, and minerals from this source were calculated.

All silos were opened approximately three months after filling and emptied over a 16-day period. This procedure ensured that experimental animals, used in the feeding trials, received in their diet samples of silage from various levels in the silos. Material which appeared inedible was separated from edible material, the total weights of each type of material were recorded, and duplicate grab samples were taken daily for dry matter determinations and subsequent chemical analyses. Further samples were taken from each silo at intervals during emptying, for carotene, pH, and volatile fatty acid determinations. After the removal of daily silage requirements the surface of each silo was covered with damp sacking to prevent drying out of the surface layers.

The weights of fresh edible and inedible silage removed, and analytical values, were used to calculate total losses of dry matter, organic matter, crude protein, and minerals during storage and the proportion of loss due to spoilage. Fermentation losses were calculated by deducting exudate and spoilage losses from total loss data. All losses were expressed as percentages of the totals ensiled.

Analytical Methods

Crude protein, organic matter, mineral matter, and pH determinations were made on the samples from the fresh herbage and silages according to methods recommended by the Association of Official Agricultural Chemists (1952).

The dry matter, crude protein, and residual sulphite contents of the exudates were determined according to methods described by Barnett (1951).

Carotene and volatile fatty acid analyses were performed on the silages according to the methods of Worker (1957) and James and Martin (1952), respectively.

Digestibility Trials

Two digestibility trials were conducted, one comparing the two silages made from the immature material and the other the two made from the more mature pasture. Each trial consisted of a five-day preliminary period and a 10-day collection period; throughout each trial three sheep were fed each type of silage as a sole feed, the same sheep being employed in both trials. In both trials the individual sheep either maintained or slightly increased their body weights during the collection period. Methods used in the collections of faeces, and

sampling of feeds, refuse and faeces, together with details of analyses and calculation, were similar to those outlined by Sears *et al.* (1942).

Total digestible nutrients (T.D.N.) were calculated in the conventional manner. Values for starch equivalent were calculated according to the method of Kellner (1926), using the factor of 1.91 for ether extract and subtracting 0.31 × and 0.33 × the percentage of crude fibre in the immature and mature silages respectively.

Palatability Tests

The relative palatability of the silages was determined by a free-choice test, immature and more-mature silages being evaluated separately. Five metal feeding bins, each subdivided into two compartments, were spaced at intervals in a concrete yard, and treated and untreated silages placed separately in each bin. Five milking cows were selected at random daily from a herd of monozygotic twins and admitted to the yard after milking where their preference for the silages was recorded. No quantitative determinations of silage intake were made. Preference was determined according to which silage the animals settled down to consume. Totals of 27 and 20 individual cows were subjected to test in the first (immature silages) and second (more mature silages) trials respectively.

RESULTS

Botanical Composition of Herbage

The botanical composition of the pasture prior to each cutting is shown in Table 1. In both cuts rye-grasses and red clover were the dominant species.

TABLE 1. BOTANICAL ANALYSIS OF HERBAGE ENSILED

Species	Percentage Composition (Dry Weight Basis)	
	1st Cut Immature	2nd Cut More-Mature
Lolium species	53.0	64.0
Trifolium repens	13.0	5.0
Trifolium pratense	26.0	28.0
*Other grasses	2.0	2.0
**Other species	6.0	1.0
Total	100.0	100.0

* Other grasses include *Holcus lanatus, Poa trivialis* and *Anthoxanthum odoratum.*

** Other spp. include *Rumex* spp. and *C. arvense.*

Chemical Composition of Herbage and Silages

Mean analytical values for the green material ensiled and silages are shown in Tables 2 and 3.

TABLE 2. MEAN CHEMICAL COMPOSITION OF HERBAGE ENSILED AND SILAGES (MOISTURE-FREE BASIS)

Treatment	Dry Matter Percentage		As Percentage of Dry Matter					
			Crude Protein		Mineral Matter		Organic Matter	
	Herbage	Silage	Herbage	Silage	Herbage	Silage	Herbage	Silage
1st Cut (immature)								
Control	17.1	16.1	19.7	22.4	8.7	10.3	91.3	89.7
Metabisulphite-treated	17.3	17.1	20.2	21.8	8.6	9.3	91.4	90.7
2nd Cut (more-mature)								
Control	21.1	19.2	15.0	18.4	6.9	8.6	93.1	91.4
Metabisulphite-treated	20.7	19.1	14.6	17.9	7.3	8.9	92.7	91.1

More-mature silages had a higher dry matter and lower crude protein content than silages made from the first cutting, but within silages from each cutting there was little variation in these constituents. Silages consistently had a higher crude protein and mineral matter content than the fresh herbage.

Mean pH values of 4.26 and 4.05, as recorded for the untreated immature and more-mature silages, approached the level of 4.0, the widely accepted value (Watson 1939) for well-preserved silage made without additives. Metabisulphite treatment produced silages with higher pH values. Mean figures of 4.50 and 4.36, as determined for the treated immature and more mature silages respectively, showed that a reasonable amount of fermentation had occurred and that sterilisation within the mass was not, therefore, complete.

TABLE 3. MEAN pH, VOLATILE FATTY ACIDS AND CAROTENE CONTENT OF THE SILAGES
(acids as g/100g fresh silage; carotene mg/100g silage)

Treatment	pH	Acetic Acid	Butyric Acid	Propionic Acid	Carotene
1st Cut (immature)					
Control	4.26	0.76	0.16	0.11	1.50
Metabisulphite-treated	4.50	0.53	0.13	0.01	2.50
2nd Cut (more-mature)					
Control	4.05	0.56	0.03	0.08	0.64
Metabisulphite-treated	4.36	0.67	0.03	0.07	2.32

Moderate amounts of volatile fatty acids were present in all silages, there being no marked differences between treated and untreated material within each cutting. Acetic acid was always present in the greatest amount, but within the range for well-preserved silage made by other techniques (Watson 1939). Although metabisulphite silages had pH values well above the critical point for volatile fatty acid formation, the low level of these constituents present suggests that pH value is of little use in assessing the value of silages treated with the additive.

At both stages of cutting, metabisulphite treatment of the herbage gave silage with a higher carotene content than that of the control. However, sufficient carotene was present in both untreated silages to supply the requirements of dairy cattle within moderate rations of silage.

Temperatures Recorded in the Silages

Table 4 gives the major temperature changes, as recorded with a potentiometer, which occurred in each silage during storage. As the upper thermo-couple in the immature treated silage was damaged, no values were recorded.

TABLE 4. MEAN TEMPERATURE IN THE SILAGES (°F)

Days After Filling	1	2	3	4	10	25	31
1st cut (immature)							
Control *	**89**	86	87	72	64	63	63
**	**107**	99	96	82	66	63	63
Metabisulphite-treated *	73	**75**	73	72	72	65	64
2nd cut (more-mature)							
Control *	76	**77**	76	75	71	67	65
**	88	92	**94**	94	89	77	74
Metabisulphite-treated	**73**	73	72	71	69	65	64
**	82	88	**91**	91	85	75	67

* 2 feet from base of silo.
** 4 feet from base of silo.
Maximum temperature in bold type.

Maximum temperature was reached quickly in all silages, immature untreated material having the highest value (107°F). Metabisulphite silages, compared with their controls, showed a lower rise in temperature —maximum temperatures recorded two feet from the base of the silo being 14°F and 4°F lower in the immature and more-mature treated silages respectively. Although temperature difference was most marked in the immature silages, this may have been affected in the untreated material by the filling procedure used. If more rapid filling had been adopted the temperature difference between treatments may have been less marked. When the silos were opened there was no evidence that silage above the upper thermo-couple in each silo had been subjected to an undesirably high temperature.

Nutrient Losses During Storage

The percentage total losses of the various constituents incurred by each silage during storage, and the portion of loss due to seepage, spoilage and fermentation, are set out in Table 5.

Total dry matter losses were high for both untreated silages, approximately one-third of the dry matter ensiled being lost during storage. However, the losses were lower than the majority of those reported in other New Zealand trials for silages made in concrete silos without additives (Sill and Sears 1942; Sears and Goodall 1947). Un-

TABLE 5. SEEPAGE, SPOILAGE, FERMENTATION AND TOTAL LOSSES OF NUTRIENTS FROM THE SILAGES
(values expressed as a % of total ensiled)

	Dry Matter				Crude Protein				Mineral Matter				Organic Matter			
	Seepage	Spoilage	Fermentation	Total	Seepage	Spoilage	Fermentation	Total	Seepage	Spoilage	Fermentation	Total	Seepage	Spoilage	Fermentation	Total
1st Cut (immature)																
Control	3.0	3.2	28.6	34.8	3.8	3.9	17.9	25.6	9.3	4.6	8.5	22.4	2.4	3.1	30.5	36.0
Metabisulphite-treated	5.8	2.7	19.6	28.1	8.9	3.0	11.3	23.2	17.1	3.8	1.6	22.5	4.8	2.6	21.3	28.7
2nd Cut (more-mature)																
Control	0.5	6.3	25.3	32.1	0.9	8.4	7.5	16.8	2.3	7.8	4.9	15.0	0.3	6.2	26.9	33.4
Metabisulphite-treated	0.9	6.2	23.6	30.7	1.7	8.4	5.2	15.3	4.3	9.3	2.2	15.8	0.6	5.9	25.6	32.1

treated immature silage had the greatest percentage total loss of dry matter, organic matter and crude protein (34.8%, 36.0% and 25.6% respectively).

Metabisulphite was effective in reducing dry matter and organic matter losses from the immature silages by 6.7% and 7.3% respectively, but had little effect on the crude protein and mineral matter fractions. Application of the salt to more mature material did not result in any great saving of nutrients—total loss of dry matter, organic matter, and crude protein being respectively 1.4%, 1.3% and 1.5% lower in treated silage compared with the control.

Reduction in total nutrient losses through metabisulphite application resulted mainly from a lowered fermentation loss, the effect being most marked with immature material. Exudate losses of all constituents in both treated silages were approximately double those which occurred in the corresponding controls, an effect which tended to lower the margin of difference in percentage total loss of nutrients between untreated and treated silages. This difference was accounted for by variation in the amount of plant moisture exuded, for seepage from treated and untreated silages within each cutting was of similar chemical composition. Percentage losses of metabisulphite in the exudate were 4.7% and 0.6% of the weight of salt applied in the immature and more-mature silages respectively. The higher loss in seepage from immature material resulted mainly from the greater volume of exudate voided.

Immature compared with more-mature silages had less top and side spoilage but differences within each cutting due to metabisulphite application were of little practical significance.

Apparent Digestibilities of Nutrients

The mean apparent digestibility coefficients of the constituents in each silage are given in Table 6.

TABLE 6. Mean Apparent Digestibility Coefficients of the Silages

Constituent	1st Cutting		2nd Cutting	
	Control	Metabisulphite-treated	Control	Metabisulphite-treated
Dry matter	65.2	68.4	55.7	60.7
Organic matter	67.5	70.0	60.0	63.6
Crude protein	67.5	72.8	62.5	69.5
Ether extract	64.4	65.4	61.2	62.2
Crude fibre	65.5	67.8	53.1	59.3
Nitrogen-free extract	69.4	70.5	64.9	63.6

The main differences were in the crude protein fraction, values in the treated immature and more-mature silages being 4.7% and 7.0% higher respectively, compared with their controls. These differences were statistically significant ($P < 0.05$). The digestibility of the crude fibre fraction was also higher in the treated more-mature silage (P

TABLE 7. MEAN VALUES FOR THE TOTAL DIGESTIBLE NUTRIENT CONTENT, STARCH EQUIVALENT, DIGESTIBLE CRUDE PROTEIN CONTENT, AND NUTRITIVE RATIO OF THE SILAGES (as % dry matter)

Treatment	Total Digestible Nutrients	Starch Equivalent	Digestible Crude Protein	Nutritive Ratio
1st Cut (immature)				
Control	64.6	54.0	14.6	1:3.4
Metabisulphite-treated	65.6	55.2	15.6	1:3.2
2nd Cut (more-mature)				
Control	56.3	45.1	11.7	1:3.8
Metabisulphite-treated	58.4	47.2	13.1	1:3.4

< 0.01) than in the control sample, but in the immature silages the difference was not marked. Metabisulphite had little effect on the digestibility of the other constituents at both stages of cutting.

The greater digestibility of the crude protein and crude fibre fractions in treated silages, compared with their controls, resulted in a higher total digestible nutrient, starch equivalent, and digestible crude protein content, but differences in favour of metabisulphite within each cutting were of little practical significance. In all silages the ratio between the digestible crude protein content and digestible non-protein fractions was narrow and of similar magnitude between untreated and treated material within each cutting, a direct reflection of the relatively high digestible crude protein content of the silages.

Relative Palatability of the Silages

Of 27 dairy cows given a free choice of untreated and treated immature silages, five cows refused both, 16 showed a preference for

TABLE 8. PREFERENCE OF DAIRY COWS FOR UNTREATED AND METABISULPHITE-TREATED SILAGES

Immature Silages

	Silage Preference		
	Control	Metabisulphite-treated	Total
Number of Individuals	6	16	22
$X^2 = 4.54$	For n = 1, P = .05		$X^2 = 3.84$

More-mature Silages

	Silage Preference		
	Control	Metabisulphite-treated	Total
Number of Individuals	4	14	18
$X^2 = 5.55$	For n = 1, P = .05		$X^2 = 3.84$

treated silage, and six favoured untreated material (Table 8). With the more-mature silages, two cows refused both, 14 animals favoured treated material, and four cows showed a distinct preference for untreated silage (Table 8).

Application of a Chi-squared test (Snedecor 1955) showed that overall preference for treated silage over the corresponding control was significant at the 5% level of probability.

Visual observation indicated that overall preference could be ascribed primarily to the animals' sense of taste. All animals were observed to nose the silages about in the feeding bin, and taste both types, before making their final choice. Sense of smell, however, cannot be disregarded as a controlling factor in the choice of silages, since metabisulphite silages had less odour than their controls.

Discussion

The differences recorded between the treated and untreated silages were, with few exceptions, small. The chopping of the material, careful consolidation, sealing, roofing, and good drainage from the silos were all in favour of making a good control silage. However, the careful mixing of the metabisulphite with the herbage, the good consolidation, sealing, and roofing were all in favour of the salt having every chance to show its value. The loss of metabisulphite in the exudate was very small.

The present results supported other findings that metabisulphite increased carotene retention within the silages (Cowan *et al.* 1953). Little, if any, reduction in volatile fatty acid production resulted from use of the salt, in contrast with the results of Alderman *et al.* (1955) and Murdoch and Holdsworth (1958). However, untreated silages of the above workers were characterised by pH values well above the level of 4.0 as considered desirable for the successful preservation of untreated silage (Watson 1939; Barnett 1954), and by moderately high-volatile acid contents. In the present study both untreated silages were well preserved as judged by mean pH values and quantities of fermentation products formed. If these silages had been made less carefully, and unchopped herbage ensiled, the advantages of using metabisulphite might have been greater.

The difference in effectiveness of the addition of metabisulphite in reducing fermentation losses in immature and more-mature material is in agreement with the results of Cowan *et al.* (1953) and Bratzler *et al.* (1956). In the present study this should not have been due to differences in losses of the salt through leaching or in mixing of the metabisulphite through the mass of herbage. It seems more likely that better consolidation was achieved with the more leafy material giving less reduction of the sterilizing properties of the salt through oxidation.

Although dairy cows showed a distinct preference for the treated silage in a free choice test, the same animals, when given only untreated silages, readily consumed them. As the metabisulphite had only a slightly beneficial effect on the feeding value of the silages, the observed

preference for treated silage in the tests appears to be of little economic importance. The results did show, at least, that metabisulphite did not adversely affect the palatability of the silages relative to silages made without additives.

In general, it is concluded that under the conditions of the trial the small saving in digestible nutrients, which resulted from metabisulphite application to immature herbage, did not justify the cost and trouble of using the additive and that no advantage was gained by applying it to more-mature herbage. However, these results must be interpreted with caution. In the present study chopped herbage was used. As several workers claim that chopping alone can favourably influence the quality of silage made from immature herbage (Woodward and Shepherd 1938; Martin and Buysse 1953; Murdoch *et al.* 1955), it may be that, with unchopped herbage, metabisulphite would have shown up more favourably in reducing nutrient losses and improving the feeding value of the final product.

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